CYTOLOGY OF THE ALIMENTARY TRACT AND ASSOCIATED GLANDS OF THE DOMESTIC FOWL (GALLUS DOMESTICUS).

by

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CONTENTS.

I. INTRODUCTION.

In spite of the great advances made in recent years in various aspects of cytology, there is still little information regarding the components of the cytoplasm and the many complicated processes, which take place in the animal cell. Much work must be undertaken and many new fields explored before the numerous existing gaps in our knowledge, can be filled.

The aim of the present work, on the glandular cells of the alimentary tract and associated glands of the fowl, was to investigate morphological changes of the cytoplasmic components relating to different physiological phases induced by fasting and food stimuli. Observations were carried out on the mitochondria and Golgi apparatus, in an attempt to throw further light on the rôle of these cell components in secretory phenomena.

In comparison with the extensive histological studies on the alimentary tract of the fowl (Clara, 1926-27, Calhoun, 1933), there are few publications on its cytology. Most of the workers in this field were attracted by the most suitable material and concentrated mainly on observations of cytoplasmic changes during embryonic life. Of these authors, Dalton (1933) worked on the liver, Saguchi (1933) studied the Golgi apparatus of the embryonic cells in tissue cultures, using, among other tissues, grafts of intestinal epithelium and of liver. Argeseanu and

and /

and May (1938) in their work on the intestinal epithelium of domestic fowl, attempted to demonstrate a correlation between cell inclusions and the various stages of cell development. For this purpose they compared the cytoplasmic picture of the intestinal epithelium at different times of embryonic life with the corresponding fully grown cells of young chickens and adult fowl. The two latter works were based exclusively on unreliable silver nitrate impregnation for the Golgi material. Hibbard (1942) gave a summary of changes in the Golgi apparatus throughout the development and differentiation of the early embryonic stomach into the proventriculus and gizzard of the domestic fowl.

As is seen from the above short list of references, very little has been done with the aid of reliable modern cytological technique, especially for the demonstration of the Golgi apparatus. None of the papers quoted contains a complete survey of all the cytoplasmic components in any of the parts of the alimentary tract investigated.

II. MATERIAL AND METHODS.

The material used in the present work consisted of samples taken from different parts of the intestinal tract, namely, proventriculus, gizzard, duodenum, ileum, caeca and rectum as well as samples taken from associated glands, namely, salivary glands, liver and pancreas, of the domestic fowl (Gallus domesticus).

Material was obtained from young chickens aged between three weeks to three months old. All were Brown Leghorns kindly supplied by Dr. W.A. Greenwood from the pure-bred stock of the Institute of Animal Genetics, Edinburgh University.

Young chickens, battery reared, of uniform breed and management and free from diseases were chosen in order to ensure the best possible conditions under which investigations could be carried out. Both sexes were used, but the majority were males. All specimens were killed by twisting the neck, the carcase was opened immediately and very small pieces of the tissues were dissected out as soon as possible, usually not later than one quarter of an hour after death. The samples were then placed in one of the fixing fluids. Series of tubes were used for each specimen.

At least two birds were used to investigate each physiological phase. When the secretory cells were found to be active, additional specimens were used as a check to avoid any mistakes due to technical errors. As a/

As a starting point for this work, birds were chosen after 24 hours fast with free access to water. Observations on all subsequent phases were undertaken at various times after feeding. Each feeding experiment was preceded by 24 hours fast, after which food was given freely, the time of the meal being noted. Food consisted of a well balanced mash with a small addition of finely grated corn mixture. The first specimens were killed half an hour after feeding and the next specimens, at hourly intervals, finishing at six hours after the food intake. The last specimens killed were those with a constant access to food without previous fast.

Generally at least two different cytological methods were used simultaneously both for Golgi and mitochondrial preparations. The best results for mitochondria were obtained with Regaud's formalin potassium bichromate, the tissues being fixed in a refrigerator. Second to Regaud, was Meves' mixture according to the following formula: 0,5 % Chromic acid in 1 % Sodium chlorate solution- 15 cc, 2 % Osmic acid - 3 cc. A slight modification in the fixing procedure was introduced - the fixing fluid was changed after 4 - 5 hours and further fixation continued for 48 hours. Other methods tried were Schriddes, Flemming and Champy-Kull, but with less constant success. For Golgi preparations, Mann-Kopsch method, with a few modifications gave most constant results, but Kolatchev as well as Sjöval and silver methods, (in spite of much less satisfactory /

satisfactory and constant results), gave a good and useful control of Golgi impregnations. In case of overimpregnation, bleaching was carried out with turpentine, or combined Potassium permanganate, or Hydrogen peroxide-turpentine method. Investigations were chiefly based on material embedded in paraffin. The majority of the sections were cut at 5 in thickness; some were at $2\frac{1}{2}$ and other at 7 in thickness. Supravital staining with neutral red and Janus green was carried out only to confirm the results obtained with fixed material. For the demonstration of fat, tissues were embedded in gelatine and frozen sections were cut. For general histological purposes Bouin, Zenker and Zenker-formalin were the most useful fixatives. Golgi preparations as a rule were mounted unstained or, in a few cases, counter stained after bleaching, with Altmann's acid fuchsin and differentiated in 90% alcohol instead of picric acid. Staining methods for mitochondria included, Heidenhein's iron haematoxylin, Regaud's haematoxylin which was the best for Regaud preparations, Altmann's acid fuchsin picric acid, and Bensley's acid fuchsin light green. Of the histological stains, Southgate's mucicarmine was most commonly used to demonstrate mucus. For the silver impregnated material, Ehrlich's haematoxylin was used as counter stain.

III. ACKNOWLEDGEMENTS.

I wish to express my thanks to Professor James Ritchie for granting me facilities to carry out this study, and for supervising my work.

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My thanks are due to Dr. W.A. Greenwood for kindly providing me with the material for this work.

IV. OBSERVATIONS.

1. PROVENTRICULUS.

A. Historical.

Cytological interest in the gastric epithelium dates back to Golgi's works on his "apparato reticolare interno". This author pointed out that the structure of the gastric cells varies greatly according to their situation in the gastric epithelium. Golgi's preparations did not reveal the presence of Golgi material in the zymogenic cells lining the glandular crypts, but three years later Kolster (1913), using silver methods, successfully impregnated the Golgi material in these cells. Later Golgi confirmed Kolster's observations, and noted, that in contrast to the other cells the Golgi apparatus lies level with, or below the nucleus, and not between the nucleus and outer pole of the cell. Since Golgi's work much research has been carried out on various aspects of the gastric gland cells. One group of experiments was confined to the strange and unusual location of the Golgi apparatus in the zymogenic cells. D'Agata (1910) attempted to prove that there is a reversal of polarity of the Golgi apparatus of the superficial gastric epithelia following simple traumatic lesions. Giraud (1928) claimed that the equatorial situation of the Golgi apparatus in zymogenic cells is a purely mechanical occurrence, due to pressure from the secretory granules. Pollister (1938) /

(1938) concluded from his own and previous observations that the peculiar orientation of the Golgi apparatus in the zymogenic gastric cells is not with reference to the nucleus, but in relation to the zone of discharge of secretion. He stressed further that careful study of the course of the capillary at the base of these cells made it clear that the orientation of the Golgi apparatus in these, as also in other cells, is most accurately expressed by stating that it is located along a course between the blood capillary and the secretory surface.

Other groups of published papers dealt purely with the description of mitochondria (Eklöf, 1914), or with general secretory phenomena (Tschassownikow, 1927). These included a number of Chinese and Japanese authors who attributed to the mitochondria a major rôle in secretion (Okanishi, 1933, Ma, 1928, Lim and Ma, 1927, Ling, Liu and Lim, 1928). So far as the writer is aware there is only one work on the cytology of the stomach of the domestic fowl (Hibbard, 1942). This author described the changes in the form and position of the Golgi apparatus in the proventriculus and in the gizzard during embryonic development. He stated that throughout the stages of development and after incubation the Golgi apparatus was demonstrated in the epithelial cells of the regions subsequently differentiated into the proventriculus and gizzard. This cell component changes during the later stages of embryonic development /

development from parallel rods (long in the gizzard and short in the proventriculus), into a complicated network. By supravital staining, using neutral red, Hibbard demonstrated that the arrangement of neutral red vacuoles not only corresponded in position with the impregnated material, but that these granules move from a position parallel with the Golgi apparatus in the 10 days old embryo, to an infranuclear level.

B._ Methods.

It should be noted that fixatives containing osmium tetroxide demonstrated many more secretory granules than the other fixatives employed, consequently they are of little use for the study of the mitochondria of zymogenic cells, which cannot be completely evacuated. In the case of these cells Regaud is to be prefered. Regaud's haematoxylin possesses great selectivity and stains the mitochondria, while leaving most of the zymogen granules unstained. In the case of the Golgi preparations with osmic and silver methods the surface epithelial cells and also cells in the glandular neck did not present any difficulty. All attempts to obtain successful osmic preparations of zymogenic cells failed, except at the time of feeding, a few cells here and there were successfully impregnated. In all phases investigated the zymogenic cells are filled with secretory granules which strongly reduce osmic acid and obstruct the whole cellular field, thus making the study of any /

of any other details impossible.

Observations on the Golgi apparatus in the zymogenic cells were based almost exclusively on the silver impregnated material (Aoyama's and Da Fano's methods). Certainly these methods are much less delicate and do not give such a fine impregnation, or so many details, as osmic methods, but when carefully handled they may provide much valuable information.

No great difficulties were encountered with the silver methods and the Golgi apparatus was shown in cells along the whole length of the glandular canals. It was found that zymogenic cells prepared by Aoyama's method gave the best results when fixed for 4-5 hours. The difficulties encountered with osmic acid in the case of zymogenic cells were analogous to those encountered by most workers on these cells.

C. Observations.

It is necessary to give a separate description of the different types of gastric cells.

Four kinds of cells all arranged in a single layer line the simple tubular glands and the surface of the gastric lumen. The zymogenic cells in the tubular glands (Pl. I. figs. 5,6,7) differ markedly from the mucous neck cells (Pl. I. fig. 4) or the cells of the superficial columnar epithelium (Pl. I. fig. 1); the latter are very similar in their morphological outlines to those of the intestinal epithelium. Over the /

Over the short distance which separates the mucous neck cells from the zymogenic cells which line the glandular crypts, are scattered cells which cytologically resemble the outer epithelium and, in their gross morphological outlines, the zymogenic cells. These more closely resemble the chief neck cells described by Okanishi (1933) than the parietal cells of mammals. They have not been identified during the present investigations in the deeper parts of the glandular crypts, and no reference to them was found in the accessible literature on the avian stomach.

(a) Zymogenic cells.

The present description will begin with that of the zymogenic cells lining the tubular glands. There appears to be some confusion amongst various authors on the nomenclature of these cells. They have been described as main cells of the gastric glands (Ekl8f, 1914), oxyntic cells, by Chinese workers (Ma, Lim, Liu, 1927-28), and zymogenic cells, by most English speaking workers.

The zymogenic cells are arranged in a single layer which lines the simple tubular glands along their whole length. Their shape varies, depending on the degree of functional activity, from low cuboidal to the elongated columnar cells. In longitudinal sections of the gland, these cells are arranged obliquely with their long axis directed slightly towards the opening of the gland. /

of the gland. They are arranged in the rows in such a way that their distal half or more than 2/3 of the cell is not in contact with the corresponding part of the neighbouring cell; thus straight canaliculi between the lateral sides of the neighbouring cells are formed which extend from the tip of the cell almost to its basal end (Pl. III. figs. 1,2,4). This gives the longitudinal sections a serrated edge formed by the distal parts of cells bulging into the lumen of the glandular crypt. The nucleus is spherical to ovoid in shape and its position depends on the functional stage of the cell; it may lie close to the basal membrane or towards the central region of the cell. In all the phases of fasting and digestion, most of these cells are densely packed with large, spherical, secretory granules. The number of granules increases considerably during fasting; in the later stages they extend from the end next to the lumen to the basal part of the cell and form a mass of closely packed granules which greatly hinders cytological observations. After 24 hours fast, when there is a maximum accumulation of secretory granules, the cell assumes a more cuboidal form with smooth rounded outlines. The nucleus is spherical and is situated close to the basal membrane. The intercellular canaliculi are often difficult to follow, as the lateral cell membranes, bulged under the pressure of accumulated secretion, are brought into proximity with their neighbours (Pl. I. figs. 6,7 Pl. III. fig. 2). /

6,7, Pl. III. fig. 2). Commencing half an hour after the intake of food the number of secretory granules decreases considerably and with their evacuation the outline and shape of the cell becomes altered. The cell becomes thinner, elongates and assumes an angular outline. The nucleus moves closer to the central region of the cell, at the same time it assumes an ovoid shape (Pl. II. figs. 5, 10, Pl. III. figs. 3,4). The intercellular canaliculi now appear as incisions or long narrow clefts wedged between the cells; this is due to the contraction of the lateral cell borders. The evacuation of the cells, which starts with intake of food, increases markedly until three hours after the meal. This phase of the progressive evacuation of the secretory granules is shown in the material prepared for the study of the mitochondria. In these preparations unstained vacuoles appear in the place of secretory granules. From three hours after feeding the cells begin to refill with secretory granules, and return to their former shape. The number of granules gradually increases and reaches , six hours after the intake of food, the level observed in the birds with constant access to food. The accumulation of secretory granules in zymogenic cells in the birds with constant access to food is never as great as in fasting specimens.

It must be noted that only a certain number of cells is involved in the secretory process at the same /

same time, and it appears that each cell acts as an independent unit. During the first hours of digestion cells situated close together often show marked differences in shape and functional stages. Cells at the bottom of the glandular crypts appear to be least involved in secretion and contain fewer secretory granules than any of the other cells.

Golgi Apparatus.

As mentioned in the section on technique, the Golgi material in zymogenic cells was studied exclusively in material impregnated with silver. It would seem from the available literature that most of the discussions on the Golgi apparatus in the zymogenic cells, and on its peculiar topography, are based on the original works of Golgi and his contemporary Kolster (1913). Kopsch (1926) has demonstrated the presence of an osmophilic apparatus in zymogenic cells of the human stomach, but he suggested that this structure resembles capillaries. Ma and Ch'ang Liu (1927) used the silver method in their work; judging from their description, they dealt almost certainly with silver artifacts and not the Golgi material itself.

Apart from these references in the available literature none of the workers on the subject, except Hibbard (1942), gave any information on their methods and successes in demonstrating the Golgi apparatus /

apparatus in the zymogenic cells. During the present work, the Golgi material was demonstrated in cells in the entire length of the glandular canal, both after 24 hour fast and in all phases after feeding. Cells in the middle of the glandular tubule are more difficult to impregnate than those nearer the bottom and neck of the crypt, this is related to the great amount of secretory granules present in the cells of the middle part. The Golgi material in zymogenic cells consists of thick links, twisted and joined in a more or less compact manner. It always lies in the basal part of the cell exactly on the level of the bottom of the inter-:cellular canaliculi (Pl. I and II, figs. 4-7). After 24 hours fast, when the zymogenic cells are packed with secretory granules, and the nucleus is close to the basal membrane, the Golgi apparatus lies at the same level as the nucleus and surrounds it laterally so as to form an equatorial belt. The anastomosing links of the Golgi material seem to be more compressed than in actively secreting cells (Pl. I, figs. 5-7, Pl. II, fig. 4). After feeding, with the decrease in the number of secretory granules, the cell elongates and the nucleus moves towards the middle part of the cell. The Golgi material does not move with the nucleus and thus gives what all students of the theory of polarity would describe as "reversed polarity". At this phase /

phase, there is a considerable loosening of the Golgi elements towards both poles of the cell. The Golgi apparatus however, never moves from its original level (Pl. II, figs 5, 6). During the phase of evacuation (unlike any other phase) some of the material treated with osmic methods contained a certain number of cells with fairly well impregnated Golgi material (Pl. II, fig. 6).

Elongate cells, with Golgi material situated below the nucleus, show in the next phase signs which might point to the commencement of secretory activity. Small clusters of argentophilic granules are present above the Golgi apparatus in the supranuclear zone (Pl. II, figs. 6, 7). In deeply impregnated material, they are blackened and appear as a uniform mass, but in faintly impregnated material the clusters of granules are clearly shown, and the delicately brown outline of each granule is visible. This detached argentophilic material in the cytoplasm was observed in many cells 1-2 hours after feeding, after that time they were less frequent-:ly seen and were seldom observed three hours after feeding.

In spite of the fact that the silver methods give less delicate impregnation than the osmic methods, the Golgi material was impregnated at all stages of secretory activity. The clusters of argentophilic /

argentophilic granules were probably recently detached from the rest of the Golgi structure.

Due to the coarse outlines of the Golgi apparatus when impregnated with silver nitrate, it is useless to look for secretory granules in the Golgi material. The granular form of the detached argentophilic clusters makes it difficult to understand why they are visible for a short time after feeding (1-2 hours) and are not visible during any other phase. The earliest secretory granules are seen in the Golgi field in mitochondrial preparations and are solely restricted to the Golgi field (Pl. II, fig. 10). Present observations on zymogenic cells confirmed that the topography of the Golgi apparatus, at least in these cells, is not influenced by the relative position of the nucleus; it lies always at a practically unchanged level at the bottom of intracellular incisions. The apparently short-lasting reversed polarity which has aroused much discussion is produced by a free movement of the nucleus, but not of the Golgi apparatus, whose position is influenced by different agents and strongly supports Pollister's conception.

The location of the earliest secretory granules in the Golgi field, the presence of clusters of argentophilic material detached from the main Golgi substance in later secretory phases, and the changes in the topography /

in the topography of the Golgi material are certainly an inevitable sign of the participation of the Golgi apparatus in secretion.

Mitochondria.

The zymogenic cells were favourable material in which to study the relationship of the mitochondria to the secretory phenomena. Perhaps because of the difficulties and, in most cases, a failure to demonstrate the Golgi apparatus in zymogenic cells, much attention has been paid to the appearance presented by the mitochondria in different functional phases.

The majority of workers agree that mitochondria are less abundant in the loaded zymogenic cells than in the discharged cells (Eklöf, 1914, Tschassovnikow, 1927).

In conformity with Cowdry's conception, a Chinese school of cytologists published a series of papers in which, with the help of elaborate des-:criptions, they tried to ascribe to the mitochondria an exclusive or at least a major rôle in secretion (Lim and Ma, 1927, Lim and Liu, 1927, Ma, 1928, Ling, Liu and Lim, 1928).

Due to the large number of secretory granules present in the zymogenic cells detailed observation of the mitochondria is very difficult. As Regaud's haematoxylin (in Regaud fixed material) tends to stain /

stain mitochondria only, thus leaving the granules unstained, clear pictures of the mitochondria were obtained in cells from fasting and fed specimens, prepared by this method. Short rods in addition to a few long thick filaments all of the same diameter, were prevalent. The small number of granules present would rather suggest that they are cross-:sections of the elongate forms. A few swellings situated at intervals along the mitochondria were sometimes seen. In cells densely filled with granules the majority of the mitochondria were found in the basal half of the cell, but a few were also dispersed in the supranuclear zone and were visible between the secretory granules (Pl. III, figs. 1, 2). At this phase the mitochondria are arranged more or less at random, without a special polar orientation. When the cell elongates and the secretory process starts, the mitochondria become evenly distributed throughout the cytoloplasm and the majority are situated in the supranuclear zone. Polar orientation becomes strongly marked by the arrange-:ment of the rods parallel to the long axis of the cell (Pl. II, figs 10, 11). In the early phases very small and deeply stained granules (Regaud's haematoxylin) are present below the nucleus in the region where the Golgi apparatus is revealed in the silver preparations (Pl. II, fig. 10). The granules are /

are much smaller than cross-sections through the rod-:shaped and filamentous mitochondria, or through the mature secretory granules. Their size, form and location strongly suggest that they are the early stages of the secretory granules. Unfortunately, no phases intermediate between these granules and the mature granules of secretion were visible. During the stage when the detached argentophilic material was seen in the supranuclear region, (1-2 hours after feeding) it would appear that the total number of mitochondria in the zymogenic cells is considerably greater than in the cells of fasting animals. It is difficult to determine if there is a real increase or if many of the mitochondria were previously covered by secretory granules and therefore invisible until there is a considerable decrease in the number of granules in the cell. No evidence suggesting the division of the mitochondria or their direct partici-:pation in secretion was observed although the same method of preparation of material was used throughout the investigation.

(b) Mucous neck cells.

These cells are large columnar cells, cylindrical in shape, they resemble to some extent goblet intes-:tinal cells. Their glandular poles are filled with mucus formed into a goblet, but without distending the cell, as in the intestinal goblet cells. The Golgi /

Golgi apparatus, easily demonstrated by both silver and osmic methods, forms a cup-like network adjacent to the lower border of the mucous surface (Pl. I, fig. 4). Mitochondria are present as extremely fine filaments which are scarcely visible, but which are shown most clearly in material fixed according to the method of Meves and stained with acid fuchsin. Their number is always very limited and they are seen only in the subnuclear cytoplasm (Pl. II, figs. 8, 9). Secretory changes are analogous to those described in the goblet cells, with the exception that the secretory granules are extremely small and fill all the supra-:nuclear zone. They show a marked affinity for acid fuchsin, but stain less deeply with haematoxylin. These numerous granules gradually accumulate at the glandular pole of the cell where a uniform mass of mucous secretion is formed and extends toward the Golgi zone (Pl. II, fig 9). Mucus can only be demonstrated in the uniform mass; the small granules do not show the characteristic reaction for mucus. During a fast, most of these cells are filled with secretion. The elimination of mucus starts with the intake of food. The outer masses are passed out of the cell and cover all the glandular surface with the mucus, but elimination is never complete, and the lower parts are immediately re-filled with newly produced secretion. The Golgi apparatus, during the increased /

increased secretory activity, expands and increases in volume (Pl. II, fig. 1). With the accumulation of secretion it moves closer to the nucleus and becomes smaller. No noticeable changes in shape, location, or in the number of the mitochondria were observed in any of the preparations.

(c) The surface epithelium.

The surface cells bordering the gastric lumen, are of the regular, simple columnar type greatly resembling the intestinal epithelium. The nucleus is oval and lies in the middle of the cell. Numerous granules, spherical in shape, fill the supranuclear region. They vary in number, depending on the functional stage, but are always present in the cells both during fasting and after feeding (Pl. III, fig. 6). Similarly as in the mucous cells, these granules stain better with acid fuchsin than with haematoxylin.

The Golgi apparatus forms a compact network and lies between the nucleus and accumulated granules, at the glandular pole (Pl. I, fig. 1). The mito-:chondria, very minute in diameter, and nearly exclusively filamentous, are scattered more or less evenly both in the supra and infra-nuclear cytoplasm. They are few numerically especially in comparison with the intestinal epithelium (Pl. III, fig. 6). During feeding the Golgi apparatus enlarges, but not conspicuously and impregnates more strongly than in the /

the fasting condition (Pl. II, fig. 1). As early as half an hour after feeding, the number of granules decreases markedly, while simultaneously new granules originate in the Golgi zone and are scattered through the supranuclear zone. Within two hours after feeding, the accumulation of small granules close to the distal cellular margin is as dense as in the fasting condition. The minute diameter of the mitochondria makes them very difficult to observe during any of the phases investigated and no marked changes were noted.

The neck cells which separate the mucous neck cells from the zymogenic cells, differ only in shape; their outlines resemble the zymogenic cells, but they appear to be more cubical. They show exactly the same internal cytological structure as the surface epithelium, and the description of the surface cells applies equally to these transitional cells.

D._ Discussion.

The different types of gastric cells of the fowl show marked changes in the cytoplasmic components during periods of fasting and after feeding. The most striking changes were observed in the zymogenic cells while the surface epithelium showed the least changes. The mucous cells occupied an intermediate position. In all the gastric cells a maximal accumulation of secretory material was observed after a /

a 24 hours fast. Following feeding, commencing half an hour after food intake there is a gradual decrease in the amount of the accumulated secretory material. Evacuation reaches its peak about 2-3 hours after a meal, and after that time there is a gradual increase in the amount of secretion present in the cell. A complete evacuation of secretory material does not take place at any time after feeding. All the gastric cells appear to exhibit constant secretory activity with a variation depending on the intake of food and periods of fasting.

The morphological changes of the cytoplasmic components due to feeding in all types of gastric cells are much smaller than in the intestinal epithelium. This suggests that during a fast, the secretory processes are greatly retarded, but are not suppressed completely, and revive after feeding. Lin and Ma (1927), using the much more drastic methods of histamine stimulation on dogs with gastric fistula, never succeeded in exhausting the zymogenic cells to such an extent as to free them completely of secretion. With the increase of secretory activity after the intake of food new secretory granules were observed in the Golgi zone of all the gastric cells examined. During the present investigation moderate hypertrophy of the Golgi apparatus takes place. In the mucous cells and surface epithelium, the secretory granules were /

were easily followed from the Golgi material to the outer glandular pole of the cell, but in the zymogenic cells (silver preparations), they could only be traced by comparing the impregnated material with sections stained for mitochondria. A study of the Golgi apparatus of the zymogenic cells showed that its behaviour is completely different from that in the other glandular cells. According to the writer, most of the descriptions, and a definite statement by Pollister (1938) that the Golgi apparatus in zymogenic cells always lies lateral to the nucleus, appear to be inaccurate. A considerable variation in the position of the nucleus in relation to the Golgi apparatus was observed in the course of the present work. During the marked accumulation of secretory granules the nucleus moves toward the basal membrane, and is encircled by the Golgi material in the form of a vertical collar. When the granules decrease in number the nucleus moves towards the central part of the cell and leaves the Golgi apparatus behind in its former position. The stable position of the Golgi apparatus suggests that polarity, or reversed polarity in the case of these cells and perhaps also in other cells, is a purely relative question, depending on the free movement of the nucleus, whose situation in the cytoplasm appears to be independent of the Golgi apparatus. This strongly /

strongly confirms Pollister's (1938) observations that the orientation of the Golgi apparatus in these cells, and perhaps in all cells, is not a question of polarity, but that it is located along a course between the blood capillary at the base of the cell and the secretory surface.

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2. DESCRIPTION OF PLATES.

Lettering.

A.G., argentophilic granule.

A.L., acinar lumen.

G.a., Golgi area.

G.M., Golgi material.

I.C., intercellular canaliculus.

M., mitochondria.

Mc.C., mucous mass.

Mc.V., mucous vesicle.

N., nucleus.

S.B., striated border.

S.G., secretory granule.

PLATE I.

Drawings of the gastric epithelia of birds killed after 24 hours fasting; showing Golgi material.

Figs. 1, and 2 from Kolatchev preparations.

Figs. 3-7 from Aoyama preparations.

Fig. 1.- Surface epithelium.

Fig. 2.- Neck cells.

Fig. 3.- Cross-section of the mucous neck cells; showing the Golgi material and nuclei.

Fig. 4.- Mucous neck cells.

Figs. 5-7.- Zymogenic cells.

PLATE I.

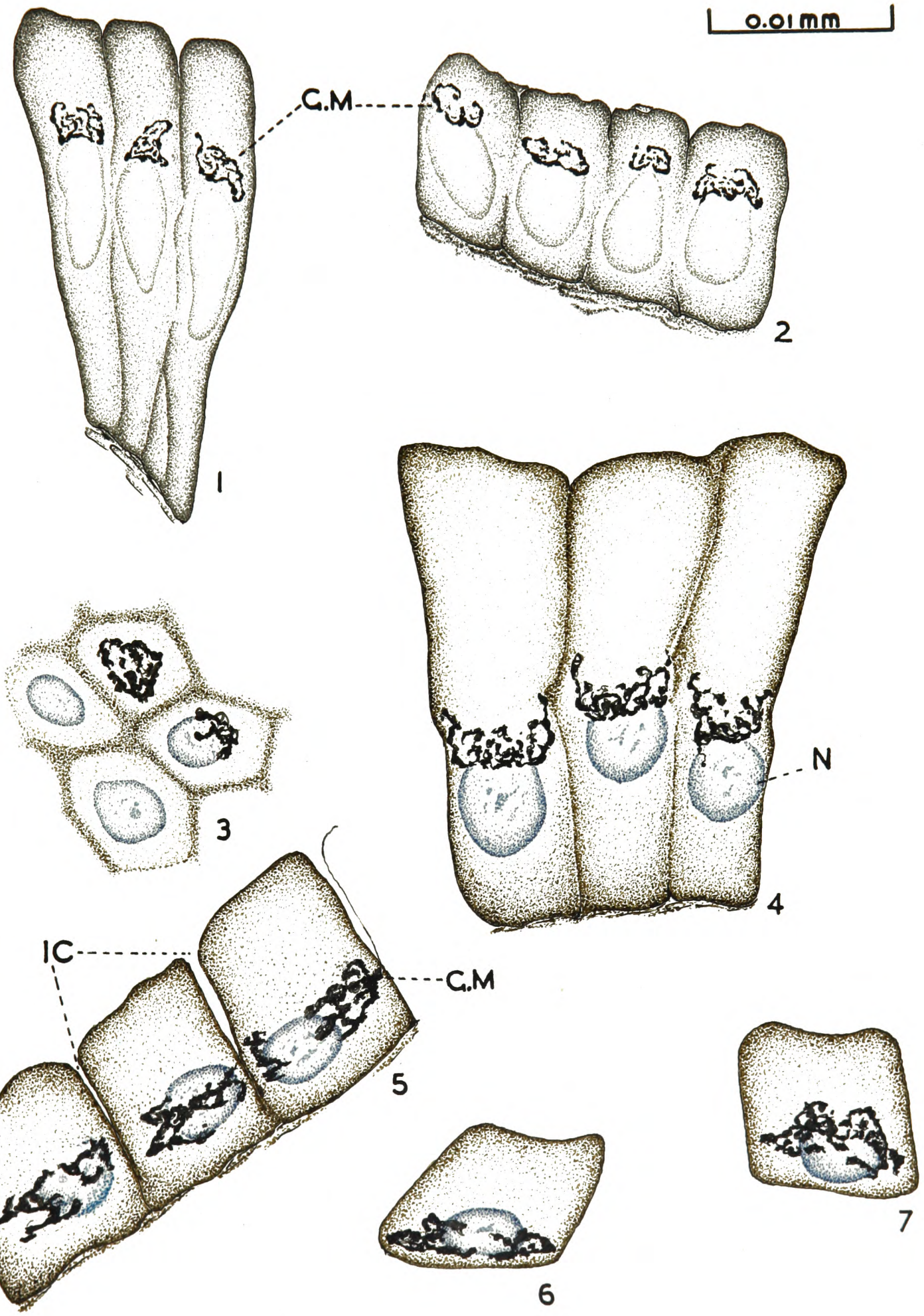


PLATE II.

Drawings of the gastric epithelia of birds killed after feeding; showing the mitochondria and Golgi material.

Figs. 1-4 from Kolatchev preparations.

Figs. 5-7 from Aoyama preparations.

Figs. 8-11 from Regaud preparations.

Fig. 1.- Surface epithelium, one hour after feeding; the Golgi material is enlarged and secretory granules are present above it.

Fig. 2.- Mucous neck cell, two hours after feeding.

Fig. 3.- Mucous neck cell, four hours after feeding.

Fig. 4.- Zymogenic cell, half an hour after feeding.

Fig. 5.- Zymogenic cell, one hour after feeding; cell elongated, reversed polarity of the Golgi material.

Figs. 6 and 7.- Zymogenic cells, two hours after feeding; argentophilic granules present in the cytoplasm.

Figs. 8 and 9.- Mucous neck cells, one hour after feeding; showing mitochondria and secre-:tory granules.

Fig. 10.- Zymogenic cell, one hour after feeding; showing, mitochondria and secretory granules below the nucleus.

Fig. 11.- Zymogenic cell, two hours after feeding.

PLATE II.

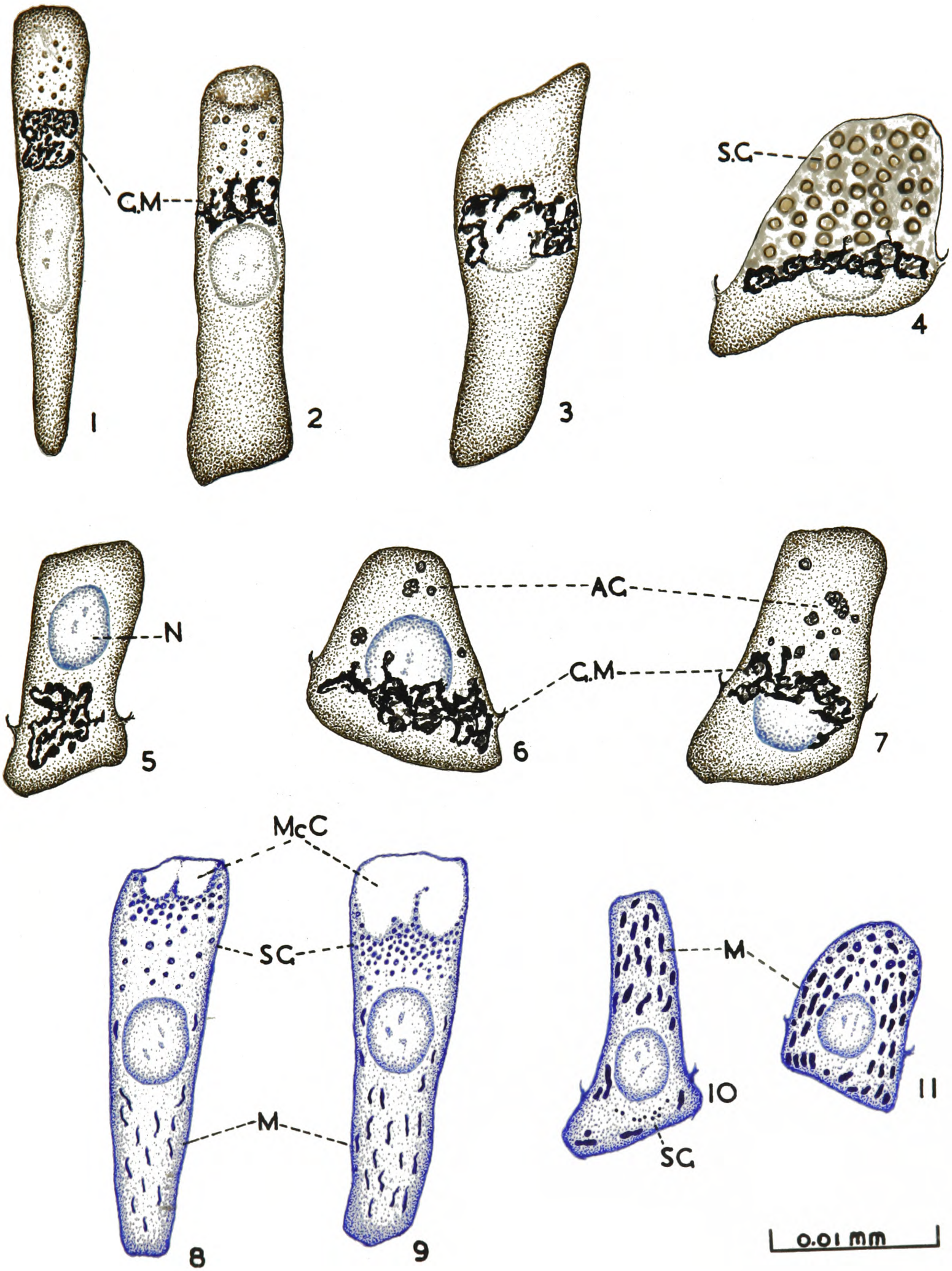


PLATE III.

Drawings of the gastric epithelia of birds killed after 24 hours fasting; showing mitochondria.

Figs. 1-4 from Regaud preparations.

Figs. 5 and 6 from Meves preparations.

Fig. 1.- Zymogenic cells from the bottom of the glandular tubule; limited number of zymogenic granules, and mitochondria well shown.

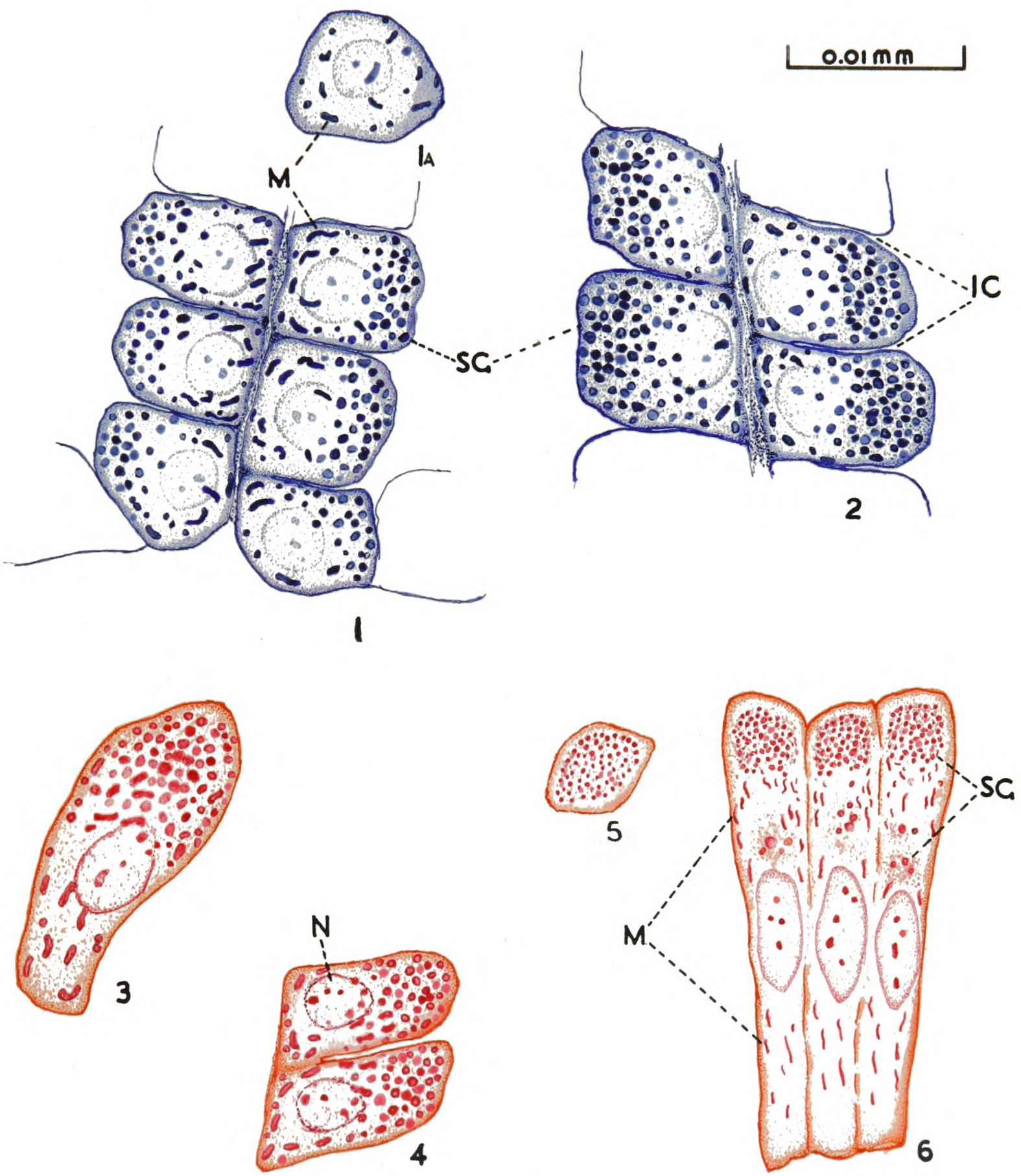
Fig. 1A.- Cross-section of the zymogenic cell; passing through the nucleus.

Figs. 2-4.- Zymogenic cells from the middle of the glandular tubule; numerous zymogenic granules shown.

Fig. 5.- Cross-section of the surface epithelium.

Fig. 6.- The cells of the surface epithelium; showing, mitochondria and secretory granules.

PLATE III.



3. GIZZARD.

The gizzard is a peculiar structure. It has a mucous membrane with a thick horn-like layer as its innermost lining. It is generally agreed that the horn-like keratinoid inner cover is a secretory product of the epithelium lining the tubular crypts. The histologists Schreiner (1900) and Calhoun (1933), and more recently the cytologist Hibbard (1942) using the Passini staining reaction demonstrated the keratinoid nature of the secretory product. No other references to the cytology of the gizzard were found by the writer in the accessible literature.

Contrary to the glandular cells of the proventri-:culus, the Golgi material of the cells of the gizzard was very favourably preserved by both silver and osmic methods. It was found, however, that in order to obtain good results with osmium tetroxide very small pieces of tissue must be used, and that the horny layer must not be large in proportion to the rest of the tissue; otherwise the highly reducing power of the keratinoid material might exhaust all the osmic acid and leave the Golgi material unblackened. To obtain the best results, the horny part was removed from some of the material and only the mucous membrane containing the glands was used. For the demonstration of the mitochondria, material was prepared by both the method of Regaud and of Meves. For the staining of secretory /

of secretory granules, haematoxylin gives much better results than acid fuchsin.

A. Observations.

The epithelial lining consists of a single layer of cuboidal cells arranged in protruding lamellae, which form simple, elongated crypts. These are filled with keratinoid secreted material connected with the rest of the outer horn-like cover. The cuboidal epithelium nearer the apices of the lamellae is more elongated and often club-shaped, with the wider part directed towards the outer keratinoid mass. A very large spherical nucleus generally fills more than half the interior of the cell, so that the latter often bulges into the lumen of the crypt. One or two deeply stained nucleoli and granules are scattered inside the nucleus (Pl. I. figs. 1-11).

Before describing the secretory role of the epithelial cells which line the crypt furrows, it is necessary to emphasize that these cells are strikingly different from other gland cells. Their peculiar behaviour suggests that they might belong to a special group of epithelia undergoing keratinization, which was first described by Deineka (1912).

Examination of the cells arranged in the lamellae showed that only cells at the bottom of crypts and the lower part of the lamellae, are normal cells /

cells in which all cell components can be easily demonstrated. The cells near to the apices of lamellae show progressive degenerative changes which finally lead to their death. In material fixed by Meves' method and stained with acid fuchsin-picric acid, whole cells embedded in the lower parts of the keratinoid mass were clearly seen. 48 hours fixation in Meves fluid is sufficient for the osmic acid to slightly darken the secretory granules which fill the cell as well as the intercellular spaces between the dead cells. These cells seem to be preserved by the secretion produced by other cells, and are infiltrated and embedded in the keratinoid mass. This keratinoid mass has its own distinct structure, consisting of numerous osmic-browned granules, dead cells and the structureless cementing mass which stains intensely yellow with picric acid. The more superficial layers of the keratinoid cover are stained a uniform yellow and do not show any structure.

A typical reticulated Golgi net is present in the cells of the lower parts of the crypts. It lies at one pole of the nucleus, or surrounds the middle region of the nucleus to form a collar-like structure (Pl. I, figs. 4,5). A few small granules are seen scattered through the net. In the epithelial cells towards the apex of the lamellae, the Golgi apparatus is smaller and gradually becomes broken up into short rods and granules /

granules (Pl. I, fig. 2). On the apices of the lamellae the Golgi apparatus was not demonstrated by any of the methods used.

Changes in the nucleus were observed in the cells situated towards the apex of the lamellae. There is a marked increase in the number of the darkly stained granules. In the vicinity of the top of the lamellae the nuclei appear to shrink and are filled with a dense reticulum.

Rod-like mitochondria, oriented along the main cell axis, are seen in the cells in which Golgi apparatus is well developed. They are usually few in number (Pl. I, figs. 8-10). Towards the apex, where the Golgi apparatus tends to be smaller, the mitochondria are few in number and stain faintly. Lastly, in the cells in which the Golgi material is beginning to break up, mitochondria cannot be demonstrated. Secretory granules in the basally situated cells are few in number and are present in the supranuclear zone (Pl. I, figs. 8-10). They gradually increase in number in the cells situated nearer the top of the lamellae. In the cells in the vicinity of the apex of the lamellae, in which Golgi material is breaking up, the granules as a rule reduce osmic acid; they assume a yellow-brownish colour like the surrounding keratinoid mass. They increase in number and fill all the supranuclear zone of the cell (Pl. I, figs. 3, 11).

Fasting /

Fasting and feeding do not induce any cytological changes in any of the epithelial cells. The formation of the granules seems to be progressive and continuous. The few granules present in the cells at the bottom of the crypt and the relatively small Golgi apparatus, which does not show any changes during the phases investigated, indicates that formation of the secretion is very slow and is not influenced by the process of digestion. The expulsion of the secretory granules must take place more slowly than their production, so that the granules gradually accumulate inside the cell. The process of secretion would appear to slow down in the older cells at the top of the lamellae, leading finally to exhaustion and death of the cell. The cell does not disintegrate, but becomes embedded in the secretory products. The disappearance of the mitochondria before that of the Golgi material in the degenerating cells, confirms the general opinion of cytologists on their greater susceptibility both to pathological conditions and to the physiological processes of senility.

Fragmentation and terminal disappearance of the Golgi material and of the mitochondria are no doubt signs of the degenerative processes taking place within the cell. The few mitotic figures seen in some of the material at the bottom of the crypts may indicate the method of replacement of the eliminated cells. As elimination /

elimination of the old cells is slow, cell division resulting in new cells to replace those worn out in the secretory process is of infrequent occurrence.

PLATE I.

Drawings of cells of gizzard; showing, Golgi material and mitochondria.

Figs. 1-7 from Kolatchev or Ludford preparations.

Figs. 8-11 from Regaud preparations.

Fig. 1.- The transverse section of the glandular tubule.

Fig. 2.- Cell close to the top of the lamellae in which the Golgi material is in form of short rods and granules.

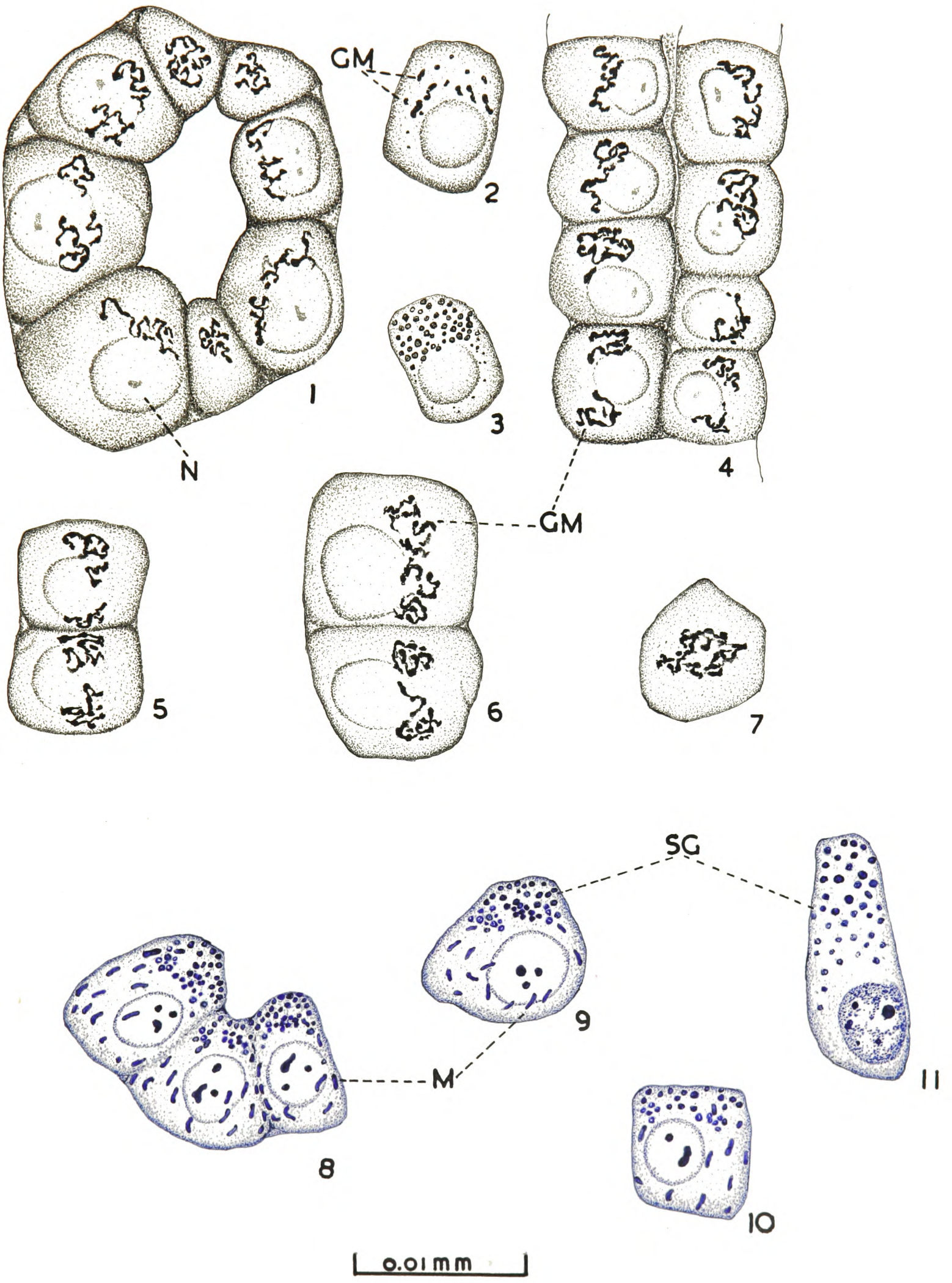
Fig. 3.- Cell in which secretory granules only are impregnated.

Figs. 4-7. - Cells from a longitudinal section of the lamellae.

Figs. 8-10.- Cells showing mitochondria and secretory granules.

Fig. 11.- Elongate cell close to the top of a lamellae; it is filled with secretory granules.

PLATE I.



4._ _INTESTINAL EPITHELIUM._

A._ _Historical.

The changes which take place in cells during digestion and absorption were the subject of many investigations, which include a wide variety of material ranging from the simplest protozoa to the complicated intestinal epithelium of the mammals. As the intestinal epithelium is easily stimulated and observed in various functional stages, it has attracted many workers who have studied it under various condi-:tions and many different aspects.

In addition to works dealing with some particular cell component of the intestinal epithelium, such as the mitochondria (Eklöf, 1914, Liu, 1930, Miller, 1922, Saito, 1933, Williams, 1943), the Golgi apparatus (Subramaniam, 1938), or the secretion of certain groups of intestinal cells, such as goblet cells (Bowen, 1926, Florey, 1932, Duthie, 1933) and Lieberkühn cells (Sawada, 1935), there is a considerable number of papers which give a more or less general review of cytological studies. These deal with the digestive tract under certain specified, or natural conditions. Some of these works are restricted to a morphological description of the cell components in particular animals, (Parascaris equorum and Toxocara canis, Argeseanu, 1934, Triton cristatus, Hermanowa, 1932, and Melanoplus differentialis and M. rubrum, /

M. rubrum, Woodruff, 1933-34). Another group of papers deals with the cytology of the intestinal epithe-:lium during fasting and digestion. Gresson (1934) worked on the mid-gut and hepatic caeca of Periplaneta orientalis, and Jacobs (1929) on the secretion of the mid-gut of Astacus leptodactylus. Kaywin (1935) studied the cytology of the digestive tract during some of the stages of metamorphosis of the bullfrog (Anura). He administered thyroid in order to accelerate metamorphosis. Cramer and Ludford (1925), and Weiner (1928) gave a detailed description of the cytological changes in the intestinal epithelium during fat absorption in rat, mouse frog and axolotl. Corti (1926) described the intestinal epithelium of the dog, mouse and man during fasting and after feeding, and observed that vacuoles appear in the Golgi field soon after the intake of food.

Some cytological data on the mitochondria and Golgi apparatus of the various cells which compose the lining of the intestine of birds were given by Clara (1926-29). He also gave a detailed histological description of the intestinal epithelia. Argeseanu and May (1938) in their work on the epithelium of the domestic fowl outlined, as the principal aim of this work, similarities and differences which exist between the cell components at various stages of development. They compared the morphology of the cell /

cell components at various phases of embryonic life with the fully grown cells in young chicken and adult fowls. They pointed out that the intestinal epithelium of the domestic fowl is very suitable material for cytological work, and this is confirmed by the present writer. The technical difficulties in demonstrating the cytoplasmic components are not great, and after the preliminary experiments preparations of uniform character may be obtained with comparative ease.

B. Methods.

Samples were taken simultaneously from different parts of the intestines of each of the birds, usually from duodenum, ileum, caeca and rectum and, in few cases, from the cloaca. After the first changes in the morphology of the cell components, following administration of food, were observed, further samples were taken from other fowl. In each case the samples were taken from the parts of the intestine situated a short distance above and below the line marked by the descending bowel contents. Material fixed according to the method of Regaud was stained satisfactorily with Regaud's haematoxylin and with Bensley's aniline acid fuchsin and light green. Schriddes' method for mitochondria and Meves' fixative proved useful; the latter was used as a routine method second to that of Regaud. Kolatchev's method in its original formula, as well as Nassonov's modification and the various modifications /

modifications of Hirschler, very seldom gave successful impregnation of the Golgi material especially in the inactive phase. On the contrary these methods tended to impregnate the mitochondria, and were used in the comparative study of mitochondria. Prolonged impregnation by Kolatchev's method at a high temperature tended to render the delicate tissues very brittle and conse-:quently lessened the chance of bringing the section through the subsequent stages without damage. Mann-Kopsch proved to be the most constant and successful method for the Golgi material, but in some cases Sjöval's formalin method also gave good results. Ludford's modification was used successfully, but, as with Kolatchev, this method made the tissues very brittle. In order to complete reduction, Mann-Kopsch and Ludford material was removed from the osmium tetroxide and placed in distilled water at 37° C for two days.

C. Observations.

As regards histological classification and description of the intestinal epithelium there is practically nothing to add to the detailed works of Clara (1926-27). Cells of very different types enter into composition of the intestinal lining. They are arranged in a single columnar layer. Each type will be dealt with separately in a later section. Beside the main epithelial cells, with epithelial cells of the Lieberkühn crypts as their variant, and goblet mucous /

mucous cells, two kinds of cells i.e. chromaffin and Paneth cells are present, but are less numerous than the other types. The functions of Paneth and chromaffin cells are still obscure. As the present observations did not provide much information concerning these two categories of cell, they will be dealt with very briefly. The main epithelial cells are referred to in this work as epithelial cells. They are the most numerous types. The goblet cells are scattered between the epithelial cells, and are second in numerical order. The numerical proportion of these two kinds of cells varies greatly. The number of goblet cells increases gradually from the duodenum to the rectum where they are nearly always predominant, and in some cases may form the entire intestinal lining to the exclusion of epithelial cells. The number of goblet cells present in a particular region, depends on the physiological phase; they increase during a fast and during the later stages of digestion, but diminish considerably in the first hours after the intake of food. The number of goblet cells also varies between the villi and crypts of the same part, being more numerous in the crypts and diminishing towards the top of the intesti-:nal villi.

(a) Epithelial cells.

The shape and size of these cells vary, depending on their situation and on the contraction of the /

of the intestinal villus. The nuclei are oval, with slight deviation in their long axis. They are situ-:ated in the basal half of the cell, but are usually closer to the middle of the cell than tha basal pole. The nuclei show a marked tendency to adapt themselves to the position of the nuclei of neighbouring cells. They are arranged alternately on two levels and thus form two rows of nuclei (Pl. IV, fig. 1). The nuclear membrane is clearly visible. The outer pole of the epithelial cell has a characteristic structure known as the striated border, a well marked, girdle-like cell extension of uniform texture with delicate longitudinal striation; this region is free of cyto-:plasmic components. The width of the striated border diminishes progressively from the anterior to the posterior part of the intestinal tract, and cannot be traced in the cells of the Lieberkühn crypts. In general, the height of all intestinal epithelia diminishes at the same rate and manner as that descri-:bed above.

The cell membranes are very faintly marked, and the cytoplasm appears to be homogenous. The corres-:ponding epithelial cells in the Lieberkühn crypts differ from those in the villi, both morphologically and in their staining properties. In proportion to the size of these cells, the nuclei are large, and are situated close to the basal membrane of the cell (Pl. IV, fig. 6). /

IV, fig. 6). The cytoplasm has a much stronger affinity for such dyes as haematoxylin and acid fuchsin, than the cytoplasm of the cells in the villi. This property creates some difficulties in cytological work, and demands careful handling during staining and differentiation. A description of the goblet cells and an account of their secretory activities will be given later.

In addition to the typical epithelial forms, as already pointed out by Clara, a number of narrow darkly stained cells were visible. Clara gave two suggestion as to their nature. One is, that they are normal epithelial cells compressed by the expanding neighbouring goblet cells, and the second suggestion is that these cells are the goblet cells after they have extruded their content. It was observed in the present investigation that the number of these cells increased soon after feeding while the number of the goblet cells diminished considerably. They were very seldom seen between the goblet cells. This strongly supports the second suggestion that these dark cells are regenerating goblet cells. Mitotic divisions were always present in considerable numbers.

As a starting point for cytological observations cells were selected from birds killed after 24 hours fast; this was taken as a resting phase. Little can be added /

be added regarding the morphology of the resting cells to the description already given in the general outline, except that the basal half of the cell below the nucleus appears to have a more conically compressed shape than during digestion, and the nucleus is situa-:ted practically in the middle region of the cell. In material stained for mitochondria no granules other than the granular mitochondria, or the cross-sections of rod-shaped or filamentous mitochondria, were observed in the epithelial cells covering the intestinal villi (Pl. IV, fig. 1).

In the phase following the intake of food (coinciding with visible secretory changes) the cells appear to be more uniform in width. The nucleus appears to move slightly towards the basal third of the cell. A light area without any distinct border becomes visible in the supranuclear region in material stained for mitochondria. This area increases in sharpness and size during the increase in cell activity and corresponds with the area occupied by the Golgi material (Pl. IV, fig 5). These are negative images of the Golgi material, and in the rows of cells on the villus form a light zone above and parallel to the outer nuclear line. Secretory granules scattered in and above the Golgi field are visible and increase in number with the progress of secretory activity.

During the phases of digestion there are no marked changes in the shape of the epithelial cells of the /

of the Lieberkühn crypts. The cytoplasm stains more deeply in all phases. A few granules, are present during the resting phase in the supranuclear cytoplasm of most of the cells. They are too small and too few in number to detect any differences between them and the mitochondria. After feeding (usually later than in the cells on the villi), secretory granules appear in great numbers, and are markedly larger than the granular mitochondria (Pl. IV, fig. 7).

Golgi apparatus.

The observations of various authors on the intestinal epithelium of vertebrate and invertebrate animals, in conditions of fasting and after feeding, showed that the Golgi material undergoes profound morphological changes, which can only be related to the participation of this cell component in functional activities. A close proximity between the first visible secretory granules and the Golgi apparatus in these cells was observed by nearly all authors. Subramaniam (1938), in his work on the Golgi apparatus in the intestinal cells of Lumbrico-nereis, observed an increase in the number of Golgi grains during secretion, and an intimate relationship between the first secretory granules and the chromophobic region of the Golgi batonnets. Similar observations were made by Jacobs (1929) on Astacus leptodactylus. Corti (1926) in his work on intestinal material (dog, man and mouse) carried out his investigations during fasting and after feeding. /

feeding. He noted one fact which appears to have been seldom observed by other authors, the fragmentation of Golgi material soon after feeding. Liu (1930), comparing the intestinal epithelium of mice after fasting for 48 hours with that of animals fed on different foods, such as fats, proteins and carbo-:hydrates, noticed that fragmentation of the Golgi material took place simultaneously with the enlargement of the mitochondria. Cramer and Ludford (1925), in their study of the rôle of the Golgi apparatus during fat absorption in mice and rats, observed that small masses of Golgi material which are present during the fasting condition swell up and enlarge after absorption of fat, so as to form a network nearly filling the part of the cell between the nucleus and the free border. They stated that numerous globules of synthesized fat are seen in the Golgi's meshes. From their further study, Cramer and Ludford concluded that the absorption of food material, other than fat, by the epithelium is not associated with any changes in the morphology of the Golgi material. Weiner (1928), in his cytological studies on intestinal epithelium during fat absorption, used various animals, frog, white mouse, field mouse, white rat and axolotl. During absorption he observed changes in both the mitochondria and in the Golgi apparatus. The Golgi material becomes more deeply impregnated with osmic acid, and there is a thickening of the threads of which it is composed. According /

According to Weiner, vacuoles appear between the threads, but hypertrophy does not take place.

During the course of the present investigations, a tedious routine was adopted of taking samples from various parts of the intestine simultaneously in each specimen investigated. It was found that strikingly different and informative pictures could be gathered. It is necessary to emphasize that greatly different response of the Golgi material at different levels of the intestinal canal were noticed after food was administered. The duodenum and the upper part of the ileum show the quickest and most noticeable response during digestion, and are the most favourable regions for observations of changes in the Golgi material during secretory activity. After a 24 hours fast the Golgi apparatus in the epithelial cells throughout the entire length of the intestinal tract shows approximately the same morphological pattern. It occupies a median area between the nucleus and the lumen, leaving a space immediately above the nucleus free. The Golgi apparatus in this phase is very small and built up of a few thin rods and threads with a few granular swellings of variable size, usually not more than twice the thickness of the threads themselves. Rods and threads are arranged more or less parallel to the longitudinal axis of the cell. A few cross links, usually of very small diameter, connect the longitudinal bars so that the apparatus appears as a simple, elongated, reticular structure /

structure (Pl. I, fig. 1-4). The Golgi material in this phase, which is a resting phase, is most difficult to impregnate successfully. The Mann-Kopsch method is the best for this purpose, but requires over three weeks in 2% osmium tetroxide at room temperature to get sufficient impregnation. Prolonged osmication is needed for any other osmic method. Silver impregna-:tion may be carried out for the usual time, but unfortunately, due to many faulty silver deposits, may only be used as a control method. After feeding, marked changes in the Golgi material take place quite quickly and successively and parallel with movement of the food contents inside the bowel walls.

At the time when the food material reaches the duodenum (usually not more than half an hour from the intake of food) numerous granules appear in the Golgi region. These are arranged in clusters and in rows following the course of the Golgi bars which are now spread out laterally and longitudinally due to the rapid increase in the number of granules. The proximity of the numerous granules creates the illusion that fragmentation of the Golgi material takes place (Pl. II, figs. 1-6). Soon afterwards, at the moment when the mature granules begin to move away from the Golgi material and become free in the surrounding cytoplasm, the reticulate structure of the Golgi material is once again perceptible. There is an increase in the number and thickness of the threads and links /

and links which now form a complicated structure with numerous granules situated along the connecting links and between the meshes of the network. Many granules which have moved away from the Golgi zone are present above it, and the phases indicating their gradual progress and terminal accumulation below the striated border may with ease be followed (Pl. III, figs. 1-3). Observations of the consecutive phases of secretion is greatly facilitated by the fact that the first cells which respond to stimulation are those on the top of the intestinal villi, and the onset of secretion in the cells near the crypts begins correspondingly later. Consequently cells in progressive stages of secretion may be studied in a single villus. Besides small single granules much larger granules, situated chiefly above the Golgi zone, were observed. Their structure is uniformly vesicular, but a few small granules are in clusters, cemented together with a brown impregnated mass. Approximately one hour after feeding, the Golgi material of the duodenum is represented as a strong reticular structure stretching between the lateral cell borders and closely approxi-:mated to reticula in adjacent cells (Pl. III, fig. 3). Consequently longitudinal sections across the intestinal villi show a wide deeply impregnated belt situated parallel to, and above, the nuclei. It is noteworthy that the Golgi material in the neighbouring cells is always at the same level and is not influenced by the distance /

distance of the nucleus from the basal pole of the cell. Numerous granules in the supranuclear region give this part of the cell a much darker appearance than the remainder of the cytoplasm.

Analogous changes were easily followed in the more distant lower parts of the small intestine, but correspondingly later and parallel with the progress of the bowel contents. These changes are less striking towards the terminal part of the intestine, and in the lower part of the small intestine they are scarcely, if at all, noticeable. In the caeca and rectum they were not observed. Beside these profound morphological changes of the Golgi material, other and no less striking physico-chemical changes appear to take place. With the onset of secretory activity there is a marked increase in the power of the Golgi material to reduce osmium tetroxide, consequently quicker and deeper blackening of the Golgi material is a visible manifesta-:tion of these changes. The Kolatchev method, which in the resting phase showed a tendency to impregnate mitochondria and seldom gave good Golgi impregnation, proved, during the secretory phase, to have the same qualities as the Mann-Kopsch technique. Less than two weeks (compared with three during the resting phase) is sufficient for the Mann-Kopsch method to give satisfactory results. Ludford's modification tends to overdarken the Golgi zone and the region where the secretory granules are accumulated. It was noted that a sudden /

a sudden response of the Golgi material takes place in cells previously brought to the resting phase by fasting, and which afterwards come into direct contact with the food. The maximum manifestation of this stimulating action seems to be about one hour after direct contact with the contents of the intestine. From that time slow retrogressive changes set in. In the specimens which had constant access to food, the Golgi apparatus seemed to occupy a median place between the resting condition and the maximum manifesta-:tion of secretory activity, both as regards structure and physico-chemical properties.

During the examination of material impregnated by any of the osmic methods, very small (mostly on the border of microscopic visibility) impregnated granules were observed in various parts of the cytoplasm of the intestinal epithelium. A few granules were seen below the nucleus and in the nuclear zone. In some cells a small agglomeration of granules was present just below the nucleus. The behaviour of the granules in the nuclear region, where only a narrow strand of cytoplasm lies between the cell and nuclear membranes, is peculiar. They seem to move on the surface of the nucleus and are sharply outlined as dark deeply impregnated granules. The presence of granules below the nucleus in some cells suggests that their passage from the basal part of cell, towards the outer pole of the cell, is obstructed by the nucleus. In the Golgi zone they are intermingled /

are intermingled with the larger secretory granules. In each phase investigated small granules were observed; their increase in number during the secretory phase suggests that they may be the prototypes of the secretory granules which, in later phases of their growth, are intimately connected with the Golgi material. They may, however, represent the extremely small and highly refringent bodies seen in the living cytoplasm by means of dark field illumination (Claude, 1943); but this, however, is improbable.

Mitochondria.

Various investigations on the relation of the mitochondria to digestion and absorption in all classes of vertebrates have yielded divergent and non/conclusive results.

Eklöf (1914), as the result of his studies on the alimentary tract of rabbit and dog, concluded that structural variations of mitochondria in intestinal cells are greater during digestion than after a 24 hours fast, and that the mitochondria are larger and more numerous during secretory activity than in resting cells. Miller (1922) found that in the final stage of secretion in rats, mitochondria are absent from the intestinal epithelium. Saito (1933), working on physiological conditions in rats, observed that a 24 hours fast produced the most marked resting phase during which filamentous forms predominate. Half an hour after feeding, the mitochondria began to break up, and /

up, and finally only spherical forms were present. This author described what he called "primary mitochon-:dria" which he considered to be young filamentous forms. Williams (1943) studied the mitochondria of the intestinal cells of Japanese salamanders (Triturus pyrrogaster) after both fasting and feeding. In contrast to the results of most of the earlier workers, Williams stated that during digestion and absorption, as well as during fasting and inanition, all forms of mitochondria are present. He stated that the correlation between the mitochondria and the digestive activities is not merely one of shape, but of the relative number of the different forms and of the distribution of the mitochondria in the cytoplasm.

The mitochondria in the intestinal epithelium of the fowl were first described by Clara (1926), and more recently by Argeseanu and May (1938). These last two authors, describing cytoplasmic changes during embryonic life, found that during the first 6 days of development only filamentous forms are seen and are equally distributed throughout the cell. In the next period rods and granules begin to form. From the 16-th day the arrangement of the mitochondria is similar to that of mature cells.

In their distribution and arrangement, the mito-:chondria of the intestinal epithelium of domestic fowl follow the general pattern described by many investigators of vertebrate material. The anterior intestinal /

intestinal cells, where secretory response is more pronounced, were chiefly used in the present investi-:gation. In the resting phase two areas of mitochondrial aggregation were observed. One immediately below the striated border, and a second in the basal subnuclear region of the cell. The aggregation at the outer pole of the cell is denser than that in the subnuclear region. In the rest of the cytoplasm the mitochondria are not numerous and are fairly equally distributed, but are practically absent from a small area directly above and below the nucleus (Pl. IV, figs. 1,2). All forms of mitochondria are present. Wavy and slightly curved filaments of various lengths are predominant. Short rods and granules, though few in number, are also encountered. Shorter filaments and rods appear to be more characteristic of the subnuclear zone, where only a few granules are seen. Close observations show that some filamentous forms have deeper stained segments and occasional bleb-like swellings. Polar orientation of the mitochondria parallel to the long axis of the cell is already well known and has been described by most workers on the intestinal epithelium.

Soon after the intake of food and at the time which closely corresponds to the first visible secretory response of the Golgi material, the mitochondria undergo certain changes. The more deeply stained segments and bleb-like swellings disappear, and evenly stained /

stained forms are predominant. The agglomeration of mitochondria close to the striated border becomes less marked and a more even distribution of the mitochondria is observed in the supranuclear zone. The wavy lines of the filamentous mitochondria are replaced by straighter lines. Short and granular forms appear to diminish in number, but do not entirely disappear. A characteristic feature of the mitochondria in this transitory phase, which is of shorter duration than the analogous period in the history of the Golgi material, seems to be their decreased affinity for the ordinary dyes such as acid fuchsin or haematoxylin. More skill and care is needed in differentiation so as to obtain lightly stained mitochondria. It seems that Saito, describing his "primary mitochondria", referred to this phase in which the mitochondria are most difficult to stain. This transitory phase is apparently correlated in some way with the onset of secretory activity.

Secretory granules appear in the Golgi area which now contains only a few mitochondria and is marked as a light zone in material stained with haematoxylin (Pl. IV, fig. 5). The small granules visible in impregnated material could not be traced in mitochondrial preparations. The stained secretory granules assume a vesicular form with a darker outline and much lighter contents. Later (when the Golgi apparatus starts the retrogressive changes) larger vesicular /

vesicular vacuoles, very faintly stained by acid fuchsin, are seen in many cells in addition to the small secretory granules. Subsequent to this phase the mitochondria show greater variety of form and more tortuous lines, making the mitochondrial picture similar to that described for the resting phase.

Towards the posterior part of the alimentary tract, the mitochondria of the epithelial cells show greater variability; the threads are less regular and do not show such a marked polar orientation as in the duodenum more mitochondria with bleb-like swellings and granular, as well as ring-like, forms are more often encountered (Pl. V, figs. 4-6). Mitochondrial response in the lower parts of the intestine is not only slower, but decreases progressively towards the end of the small intestine.

The mitochondria in the cells of the Lieberkühn crypts cannot normally be seen unless differentiation is stopped at the moment when the cytoplasm is still of a dark shade. Only a few mitochondria are seen in the nuclear and infranuclear region. Shorter filaments are the prevalent forms in the supranuclear zone and appear to be less numerous here than in the cells in the villus. No marked changes were noted in this part of the intestinal lining.

(b) Goblet cells.

The mucous glandular cells of the intestinal epithelium /

epithelium, with their intermittent secretion (characterizing all mucus secreting cell types), attracted the attention of cytologists at an early date, no doubt because they provide easy material for study of secretory activities. Cajal (1904), was the first to describe in goblet cells the characteristic loosening and compressing of the Golgi net depending on the accumu-:lation of the secretion. He was, however,too cautious to attribute any rôle to the Golgi material during secretion. As many later workers (Nassonov, 1923, Bowen, 1924-27, Florey, 1932, and Duthie, 1933) contributed to our knowledge, the cytology of these cells is better known than that of other mucus secreting cells in general, and intestinal cells in particular.

Investigations in the course of the present work did not provide much new information on the goblet cells, and most of the observations made confirm the descriptions of previous workers on these cells in other animals.

The goblet cells of the domestic fowl differ markedly from those of mammalia. They are always in the form of a neatly shaped goblet. Their basal part, which contains the nucleus and most of the cytoplasm with its components, is narrow. The nuclei of goblet cells stain, as a rule, deeper than those of epithelial cells. The mitochondrial pattern closely follows that of the other cells, but greater changes are visible during the later phases of secretion. The Golgi apparatus /

apparatus is situated between the nucleus and the lumen and forms a reticular net, composed of rods and links of variable thickness (Pl. VI, figs. 1-7). Changes in the Golgi material during secretion are marked. In the early phases the Golgi material is present for the most part in the form of short segments and threads (Pl. VI. fig. 1). With the onset of secretory activity, the reticulum becomes much more complicated, forming a cylindrical basket which stretches throughout the whole length of the narrow part of the cell. Along the Golgi links secretory granules are visible in intimate connection with the Golgi material. Free osmophilic granules inside the basked can be traced as they change into larger, non-osmophilic vesicles which are intermingled with them. These non-osmophilic vesicles can be demonstrated by mucus staining dyes (mucicarmine, tolluidine blue, thionine) as in the salivary glands (Pl. VII, figs. 2 and 5). They make their way from inside the Golgi basket to the outer pole of the cell where they gradu-:ally fuse together to form a uniform mucous mass. This mucous mass replaces the cytoplasm and pushes the cytoplasmic components towards the narrow basal stem. The fusion of mucous vesicles to the uniform polar mass could be observed in the basal part of the goblet (Pl. VII, figs. 2 and 3).

The consecutive phases in the production of mucus could be traced by comparing Golgi preparations with material /

with material stained for mitochondria. Observations are greatly facilitated in mitochondrial material counter-stained with mucicarmine, which demonstrates very small droplets of mucus in the Golgi field. As the formation of the goblet nears completion, the secretory processes seem to diminish, and the Golgi material begins to retrogress, contracting first towards the basal part of the cell (Pl. VI, figs 6 and 7). Free secretory granules, judging from their limited number, seem to be short lived and their transformation into non-osmophilic mucous vesicles must occur quite quickly. The mitochondria, at the time of the accumu-:lation of the secretory mass in the goblet, are pushed towards the narrow basal part of the cell. They are most numerous close to the membrane separating the cytoplasm from the basal border of the mucous goblet (Pl. VII, figs. 1-7). Mitochondria were not identified between the mucous masses, as is the case in the salivary mucus secreting cells. It was not determined whether the accumulation of mitochondria on the border of the secretory material is purely mechanical, or if it has any connection with secretion. No conclusive evidence could be obtained which would suggest their direct transformation, or even partici-:pation, in the secretory processes.

(c) Chromaffin and Paneth cells.

In spite of many disputes and hypotheses, the rôle played /

played by the chromaffin and the Paneth cells in the intestinal lining remains completely obscure.

Of the cells described by histologists as chromaf-:fin and Paneth cells, only one type was observed in the material investigated. They are encountered in all parts of the intestine, but are more numerous in the crypts. Their form varies from bottle-shaped, with the narrow neck directed towards the intestinal lumen, to spindle-shaped forms laying between the epithelial cells; they always touch the basal membrane (Pl. I, figs. 8 and 9). The large lightly stained, vesicular nucleus lies in the middle part of the cell.

The cytoplasm is filled with a considerable number of granules which possess peculiar properties. In mitochondrial preparations they stain deeply and uniformly with haematoxylin (Pl. IV, fig. 8). In Golgi impregnated material they blacken uniformly with osmic acid, and as easily as the Golgi material during secretion (Pl. I, figs. 8 and 9). During fasting and at all times after feeding the cells behave in the same way and no difference in the number of granules, or in their staining properties, was noticed.

D. Discussion.

The various cells which compose the intestinal lining react in different ways during fast and feeding.

While the secretory cycle of the main cells could be easily influenced and brought to the resting phase by a short /

by a short fast, this cannot be done with the mucous goblet cells in which secretory activity seems to be governed by entirely different factors. The same kind of cells respond differently morphologically, and very probably perform different tasks in the various parts of the intestinal tract. Williams (1943) noted that the response of the mitochondria is more pronounced in the anterior intestinal cells. During the present work it was observed that this is also true of the Golgi material, and that towards the posterior part of the intestinal tract the intensity of the changes diminish gradually and disappear completely in the lower parts (caeca, rectum).

Epithelial cells brought to a resting stage by a fast become strongly sensitized to the subsequent administration of food. Observations, following the routine of taking samples from the parts above and below the line marked by the descending bowel contents, offer support in favour of the view that the secretory changes are initiated by the direct contact of the cells with the food material. This assertion is further supported by the fact that secretory changes begin in the cells at the apices of the villi, and that the cells situated lower in the epithelium react later.

The striking changes in the Golgi material in the upper part of intestinal epithelium, as soon as the bowel contents reach it, strongly indicate that the Golgi /

Golgi material plays a specific part in the secretory phenomena. It would be more than illogical to negate any participation of this cell component in these phenomena after observing and comparing material from birds killed at various phases after fasting and feeding. Whatever the rôle which the Golgi material plays in these processes, its participation is very plainly marked.

Williams (1943), basing his observations on the more difficult and less perceptible changes of the mitochondria, came to an erroneous conclusion. He stated that upon ingestion the entire digestive tract immediately becomes sensitized. He concluded further, that the later reaction of the posterior part of the intestine is because it is more concerned with absorp-:tion than with the secretion of enzymes. The present investigations show that the intensity of the reaction of the cells, and not the time at which the morphological changes begin, support the latter part of this statement. The following facts support the suggestion that the anterior part of the intestine is more concerned with secretion than any other part. Striking changes in the Golgi material of the upper part of the intestinal epithelium becomes visible as soon as the bowel contents reach it, usually in less than half an hour from the intake of food. It is difficult to imagine that in such a short time digestion could take place to such an extent as to convert the food into an assimilable /

assimilable form, and consequently that the changes in the Golgi material are due to absorption. Secondly, the progressive phases in the production of the secretory granules, clearly indicate that all these granules make their way towards the striated border of the cell. There is no indication that any of them take a different course, e.i. towards the basal part of the cell as they would do if composed of absorbed material.

Diminished morphological changes of all the cell components in the lower part of the intestinal tract would indicate that secretion in this part (except of mucus) must be limited, and that absorption does not actively influence the cell components. Sudden and short-lived changes in the mitochondria during the first transitory phase, when the cell components seem to be mobilized to the secretory processes, are an indication of their share in the synthesizing processes, which terminate in the production of free granules and their further growth.

PLATE I.

Drawings of the intestinal epithelium of birds killed after 24 hours fasting; showing the Golgi material.

Figs. 1-7 from Mann-Kopsch preparations.

Figs. 8 and 9 from Kolatchev preparations.

Figs. 1-4.- Cells of the intestinal villi.

Figs. 5-7.- Cells of the Lieberkühn crypts.

Figs. 8 and 9.- Chromaffin cells.

PLATE I.

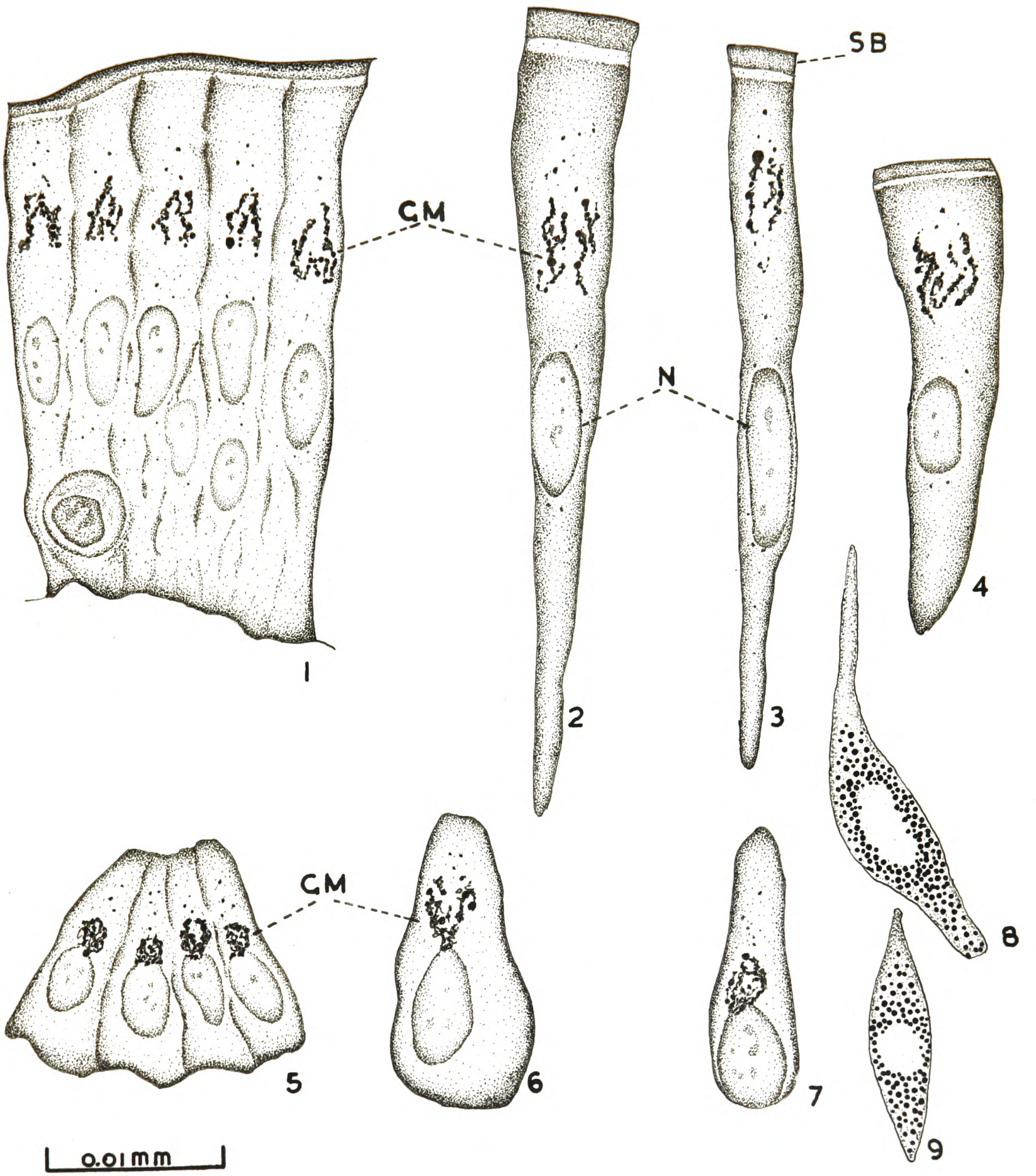


PLATE II.

Drawings of the intestinal epithelium of birds killed after feeding; showing the Golgi material.
All figures from Mann-Kopsch or Kolatchev preparations.

Figs. 1-6.- Cells on the villi, at the time when the food reaches this part of the intestine; showing fragmentation of the Golgi material and formation of secretory granules.

PLATE II.

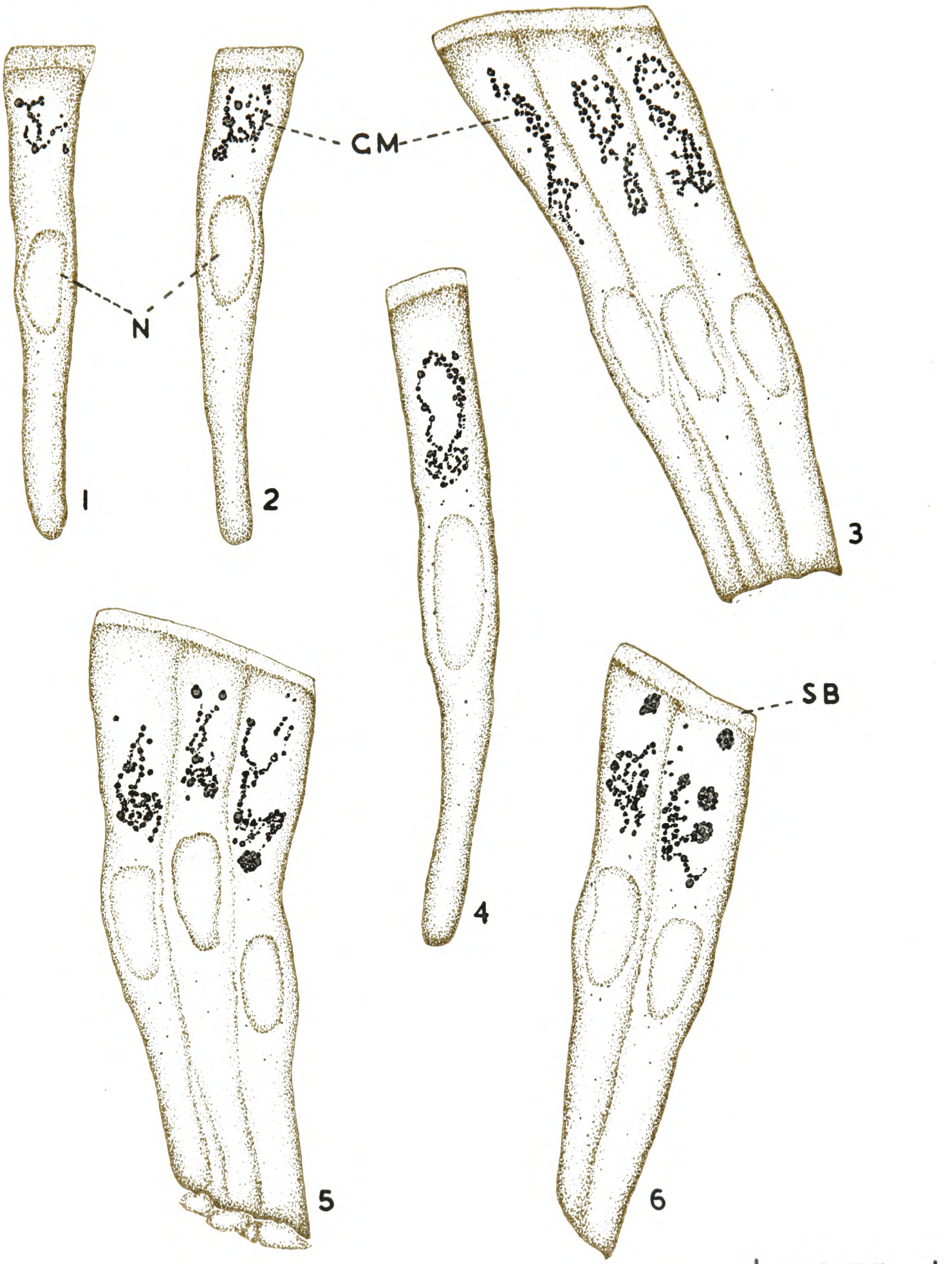


PLATE III.

Drawings of the intestinal epithelium, from the duodenum of birds killed after feeding; showing the Golgi material.

All figures from Mann-Kopsch or Kolatchev preparations.

Figs. 1-3.- Cells on the villi, one hour after feeding; showing hypertrophied Golgi material and the secretory granules.

PLATE III.

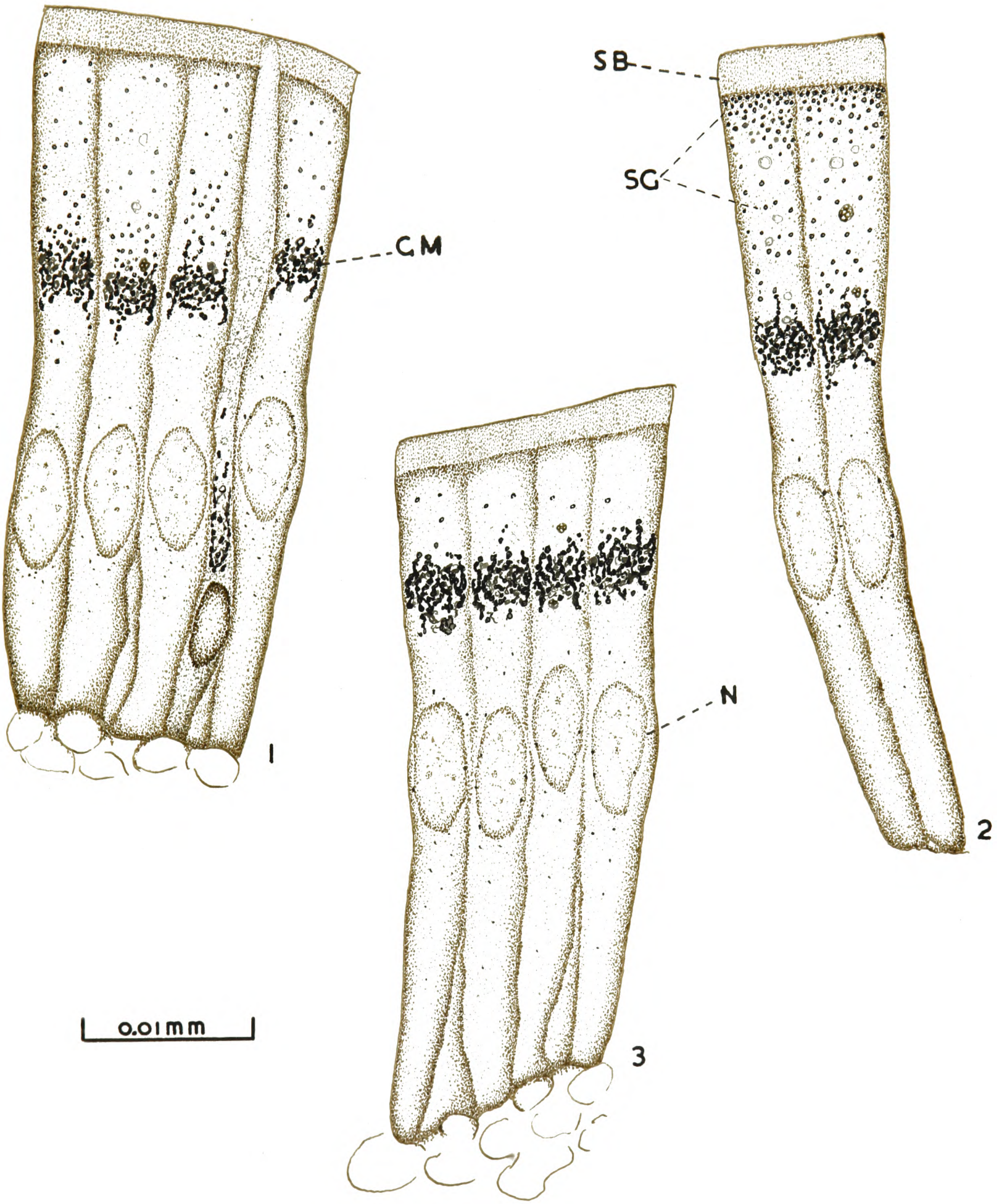


PLATE IV.

Drawings of the intestinal epithelium of birds killed after 24 hours fasting and after feeding; showing the mitochondria.

All figures from Regaud preparations.

Figs. 1 and 2.- Cells from the duodenum; after 24 hours fasting.

Figs. 3 and 4.- Cross-sections of the epithelial cells.

Fig. 5.- Cell of the duodenum, one hour after feeding; secretory granules are seen in the Golgi field.

Fig. 6.- Cells of the Lieberkühn crypts of the duodenum, after 24 hours fasting.

Fig. 7.- Cell of the Lieberkühn crypt of the duodenum, one hour after feeding; showing secretory granules.

Fig. 8.- Chromaffin cell.

PLATE IV.

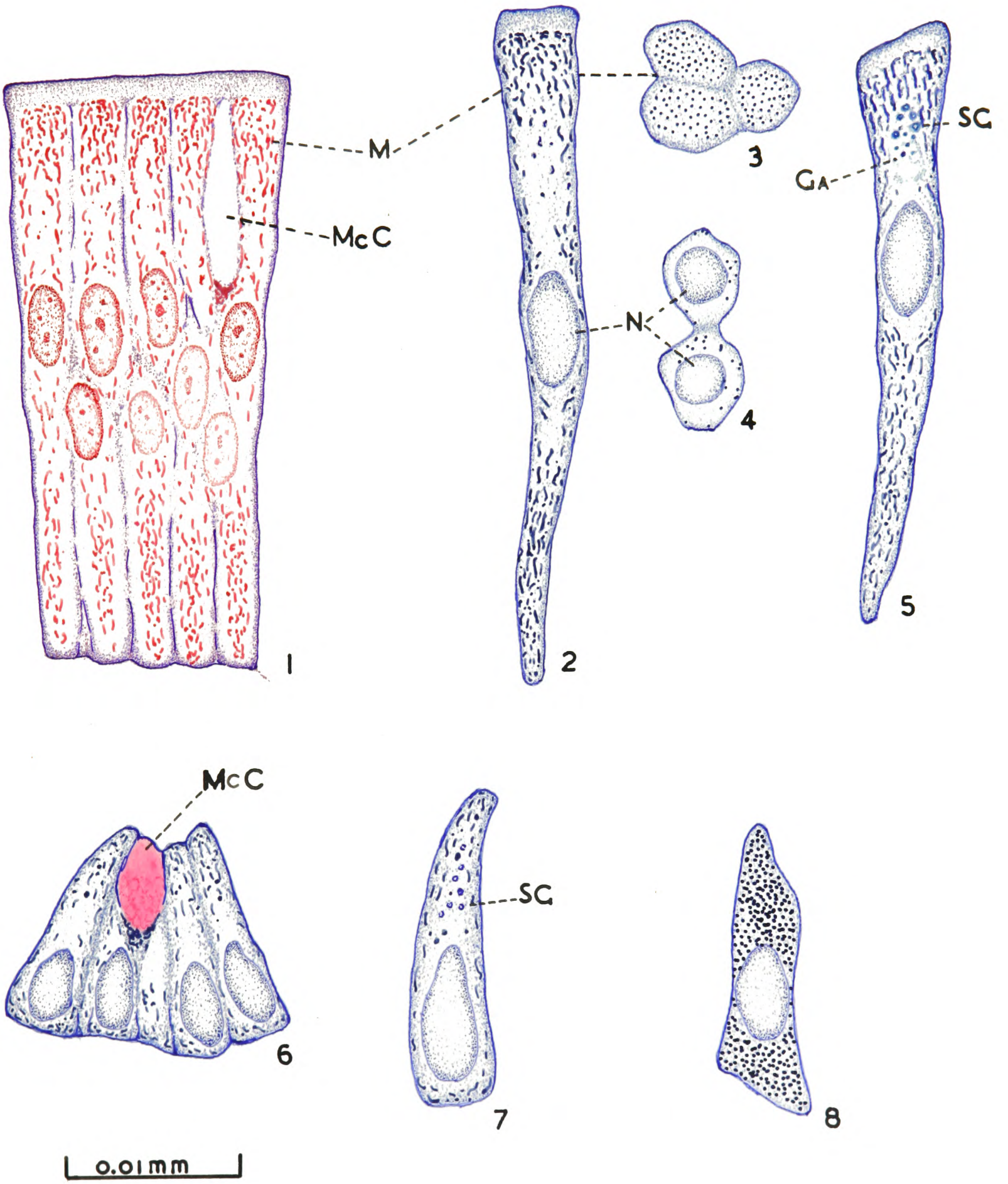


PLATE V.

Drawings of the intestinal epithelium of the lower parts of the intestinal tract (caeca and rectum) both after 24 hours fasting and after feeding; showing the Golgi material and mitochondria.

Figs. 1-3 from Mann-Kopsch preparations.

Fig. 4 from Meves preparations.

Figs. 5 and 6 from Regaud preparations.

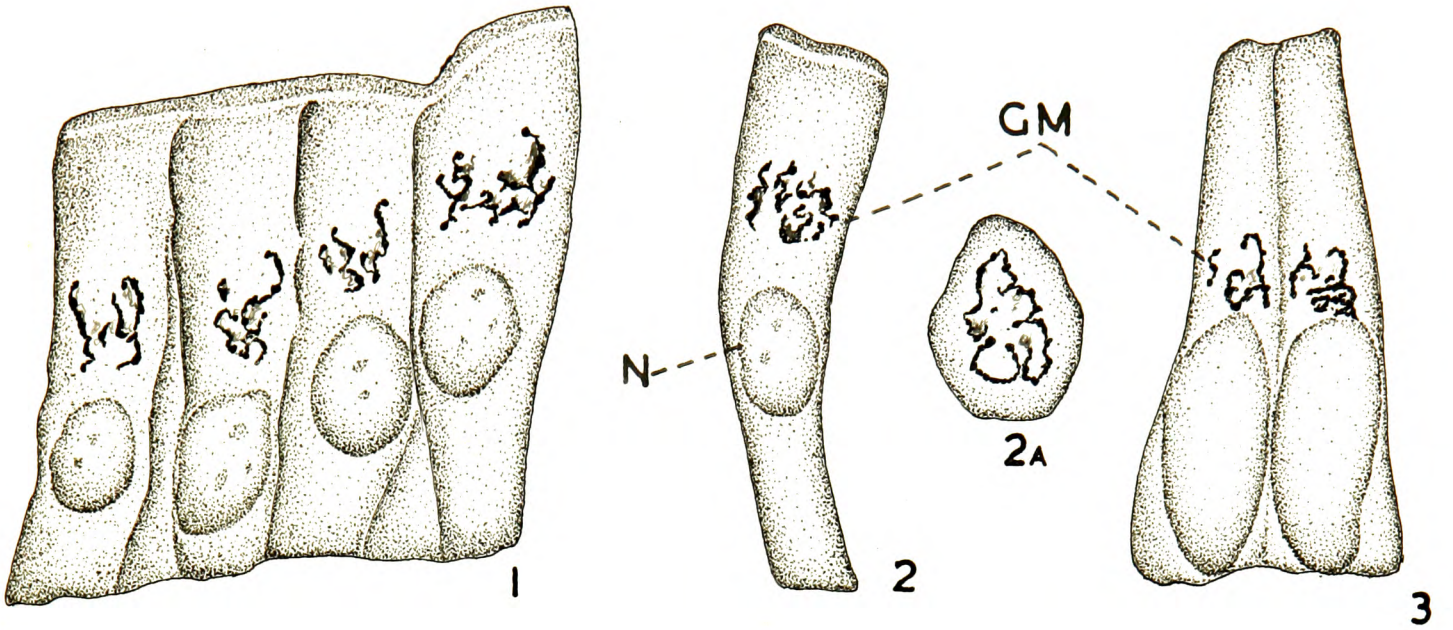
Figs. 1 and 2.- Surface epithelium; showing the Golgi material.

Fig. 3.- Cells of the crypts; showing the Golgi material.

Figs. 4 and 5.- Surface epithelium; showing great variation in the form of the mitochondria.

Fig. 6.- Cell of the crypt.

PLATE V.



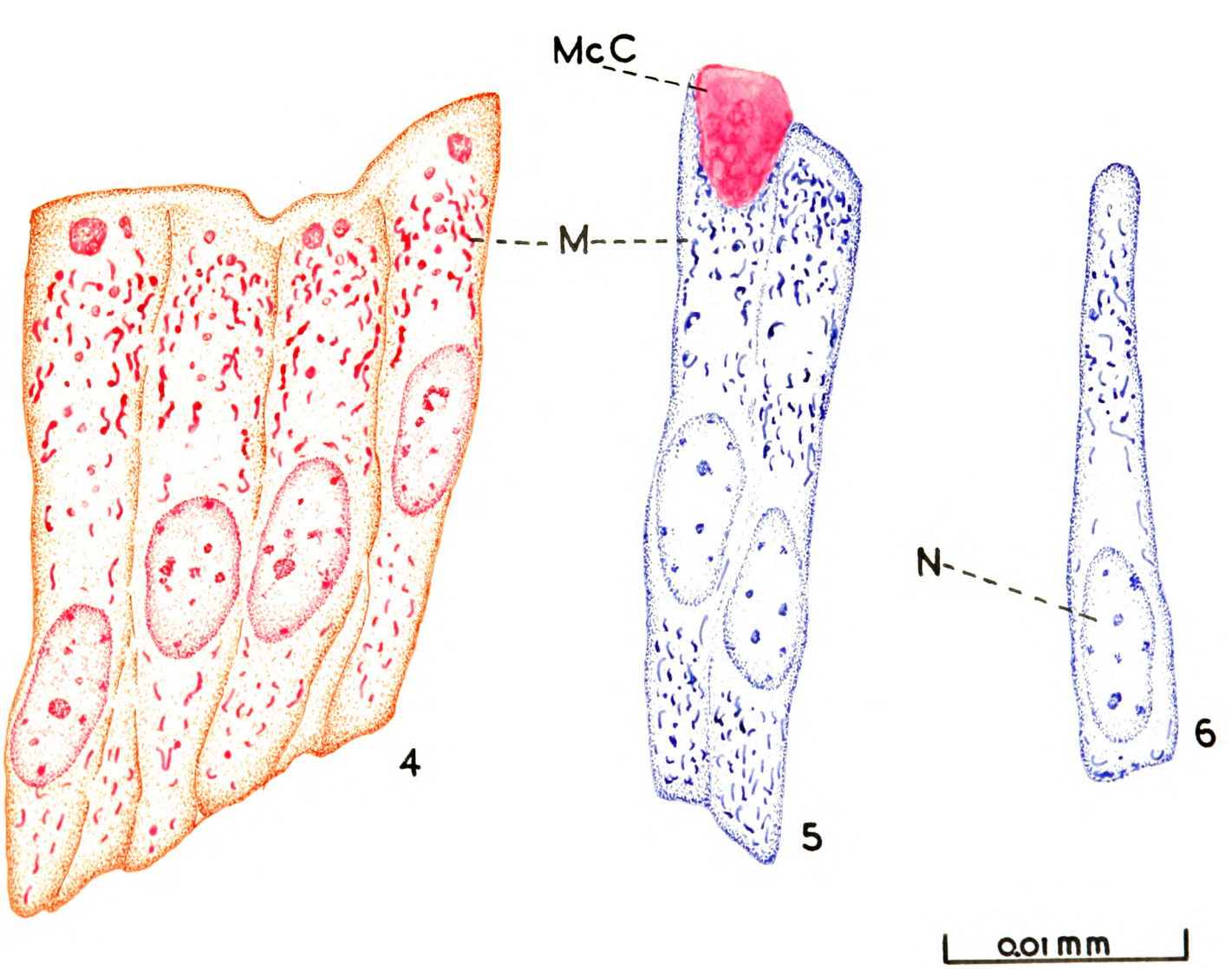


PLATE VI.

Drawings of mucous goblet cells; showing the Golgi material.

All figures from Kolatchev and Mann-Kopsch preparations; showing progressive phases of secretion and changes in the Golgi material.

PLATE VI.

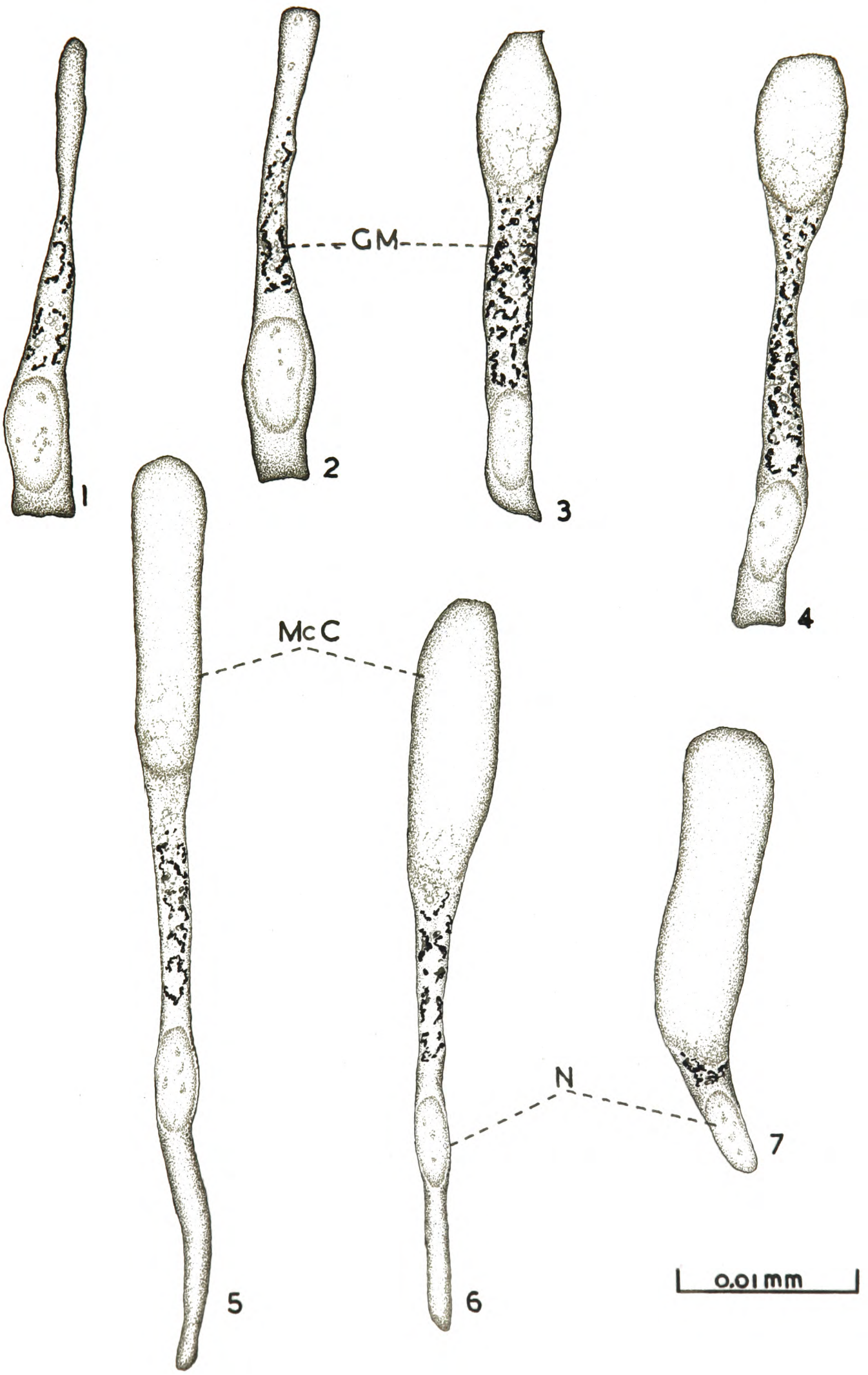
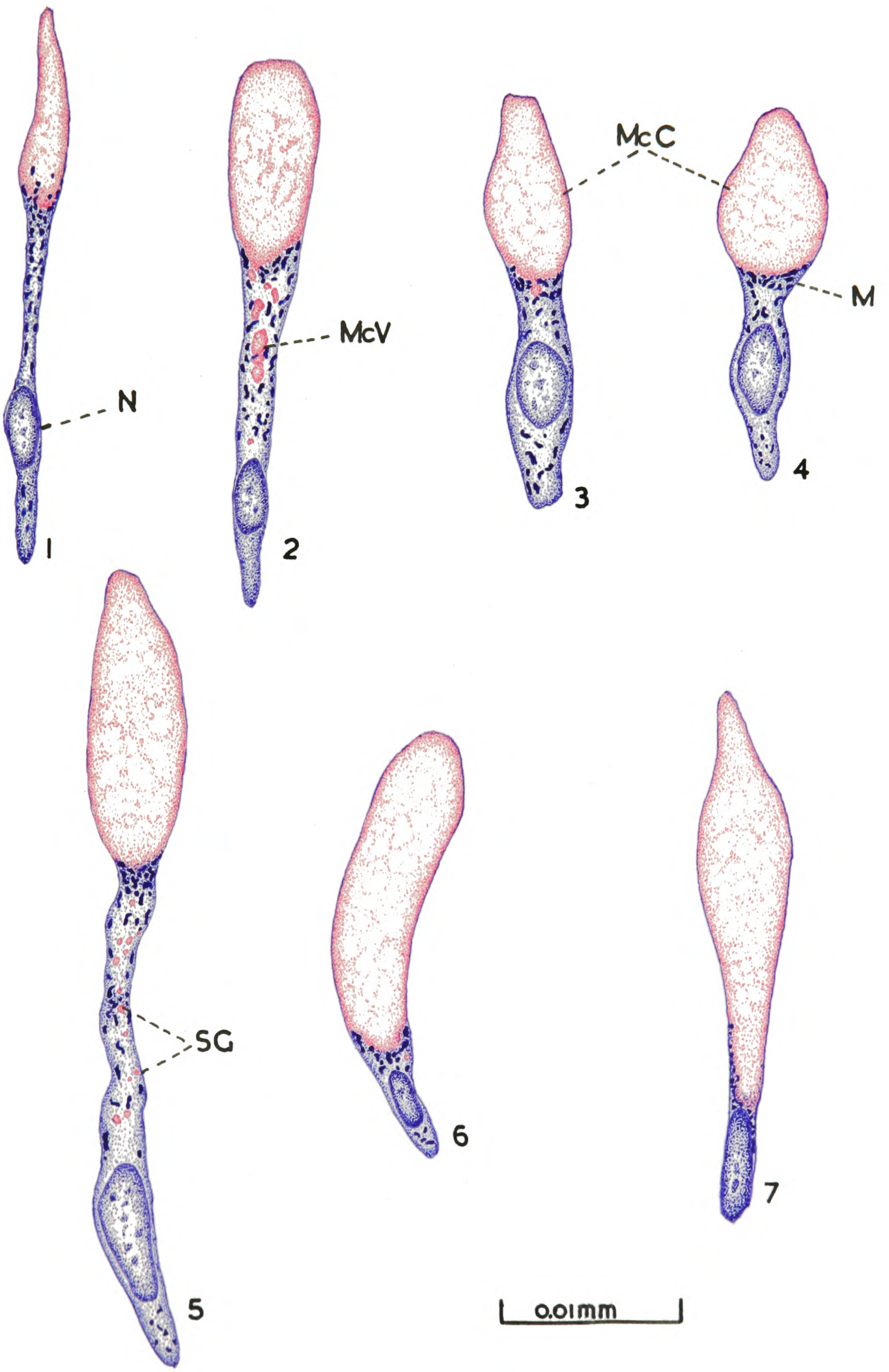


PLATE VII.

Drawings of mucous goblet cells; showing the mitochondria.

All figures from Regaud preparations; showing progressive phases of secretion and formation of the goblet. In figures 2 and 5, mucous vesicles are seen in the Golgi field.

PLATE VII.



5. SALIVARY GLANDS.

A. Historical.

Since the early stages of modern cytology, salivary glands of insects and mammals were favourite objects for cytological studies. The cytology of the so-called salivary glands of Chironomus larvae was for some time the subject of many cytological works (Beams and Goldsmith, 1930, Gatenby, 1932, Parat and Painlevé, 1925). Salivary glands of other insects were also the subject of some later papers, namely the salivary glands of the Grasshopper, Rhomelea microptera (Beams and King, 1932), and Tipula paludosa (Gresson, 1937).

Of the vertebrate animals, small laboratory rodents were second in order of popularity among other workers in this particular field (Tupa, 1926, Honda, 1926, Moley and Smith, 1930, Duthie, 1934). So far as the writer is aware, no published work has yet appeared on the cytology of the salivary glands of birds. The difficulties encountered in obtaining good cytological preparations of avian salivary glands were the chief reason why they have not been studied. Disappointing results are to be expected more often when using mitochondrial techniques than with methods for the demonstration of the Golgi material.

As to the general features of the salivary glands of the domestic fowl, it appears that, in contrast to the variety of cells encountered in the more or less differentiated /

differentiated salivary glands of mammals, there is great simplicity and uniformity in avian material. Uniform agreement exists between histologists (Heindrich, 1907, Calhoun, 1933) on the nature of the secretory material, which is purely and exclusively mucous and shows a characteristic colour reaction with certain dyes (mucicarmine, toluidine blue). Single units of the mucous glands, beside several compact anatomical agglomerations in the mouth cavity, are scattered more or less at random on the dorsal part of the crop and throughout the entire length of the oesophagus.

B. Methods.

In the first phase of this investigation, single anatomical agglomerations in the mouth cavity and samples of glands from the crop and oesophagus were dealt with separately. In subsequent works, due to their similarity, samples from all the groups of salivary glands were cut into small pieces, mixed together and immediately fixed. Bouin and Zenker were used for the preliminary histological observations. Regaud, Meves and Champy-Kull fixatives were used for the demonstration of the mitochondria. Material treated after the method of Regaud, stained with Regaud's haematoxylin and counter-stained with Southgate's mucicarmine to demonstrate mucous secretion, gave an instructive differential staining and proved to be most useful /

most useful in following the secretory processes from the early stages. Meves and Champy-Kull material stained best with Altmann's aniline acid fuchsin-picric acid. Kolatchev, Mann-Kopsch and Ludford were used to study the Golgi material; the last two, with a final reduction of the osmic acid in distilled water at 37°C for 48 hours, gave the best results for the salivary glands. The Golgi material was examined in unstained preparations, or the sections were counter-stained with neutral red (Ludford). Silver methods of impregnation were of little use in the case of this organ. In order to study the secretory granules and their relation to the cytoplasmic components, particu-:larly with reference to the Golgi apparatus, material suitably impregnated by the Mann-Kopsch method was bleached in turpentine and subsequently stained with aniline acid fuchsin. It was then differentiated in 95% alcohol instead of picric acid, which, because of a strong tendency to bleach the Golgi material, cannot be used for this purpose.

C. Observations.

The epithelium of the salivary glands consists of a single layer of cylindrical cells with their basal part resting on a thin laminar membrane. The lamina projects into the glandular cavity in the form of an elongated fold, and forms a partition which supports two rows of glandular epithelium, one on either side. This structure /

This structure gives a characteristic honeycomb appearance to each single fold. There is a considerable variation, both in size and in shape, between the cells themselves and between the various glands. These variations are correlated to a certain extent with the place which the cells occupy in a particular row, and to a greater extent with the phase of secretory activity. Often cells quite close together show marked differences. In spite of the large number of samples investigated, no mitotic figures were seen. The majority of cells examined, after 24 hours fasting or at any time after feeding, are elongated conical cylinders with very faintly marked borders. A small spherical nucleus lies close to the basal membrane. It often stains intensely and appears as a homogenous mass; in other cases it contains a deeply stained nuclear net and granules. These cells are completely filled with the accumulated secretory material which is coloured by the mucus stains. The accumulated secretory material is in the form of vesicles, probably of fluid consistency, which are divided into groups by narrow cytoplasmic strips connected together and forming a reticular structure between the vesicles. A somewhat wider strip of cytoplasm is present in the basal part of the cell surrounding the glandular side of the nucleus and extending above it (Pl. II, fig. 8). The mitochondria and Golgi material are visible inside the cytoplasmic strips. A full description of the cytoplasmic /

cytoplasmic components will be given in the next section.

After a 24 hours fast every cell in the salivary gland is completely filled with secretion. This condition was also common at any time after a meal. Feeding induces very little change in the majority of cells, but after a meal a certain number of cells (usually very limited) discharge their secretory contents into the glandular lumen, where it is easily demonstrated. Dry food without simultaneous access to water seems to stimulate more cells to discharge their contents than when water is available. Birds which had previously fasted and were then allowed free access to food for at least two hours, provided good material to study the different secretory phases. Due to the limited number of cells which discharge their contents after a meal, very careful examination of the sections is necessary in order to follow the consecutive phases of secretion. It was observed that when a cell is stimulated to discharge its contents, the process continues until it is empty of secretion. Various phases of cell evacuation were studied. Some cells were found in which the lower part had become narrow, while the cell membrane next to the lumen appeared to be ruptured, so that the top part of the cell remained widely open in bell-like fashion. In the final stage the secretion is discharged into the lumen of the gland. The presence of secretion in the lumen is easily /

easily demonstrated by staining. Following the evacua-:tion of the secretion, the cells become narrow and small (Pl. II, fig. 9).

Before they enter upon the new phase of activity, the cells pass through a transitory regenerative phase. They enlarge gradually; the nucleus slowly loses its uniform staining properties and moves from the basal part towards the middle region of the cell. Uniform homogeneous cytoplasm containing cytoplasmic structures now fill the cell, which is surrounded by a continuous cell membrane (Pl. II, fig. 1). This period of regeneration resulting in a reorganization of all the cytoplasmic components, is a preparation for the secretory activity upon which the cell will enter in the next phase. The phases of secretion are as follows: Small vacuoles, or more correctly, mucous containing vesicles (demonstrable with Southgate's mucicarmine) appear in the cytoplasm in the region between the nucleus and the lumen (Pl. II. figs. 2 and 3). The size and the number of these vesicles increases gradually, and a vesicular area develops at the glandular pole of the cell (Pl. II, fig. 4). This area, in the later stages, expands markedly towards the basal part displacing the cytoplasm which forms narrow cytoplasmic strips which divide the mucous mass into large vesicles, and a thin layer on the inside of the cell membrane. During the accumulation of the secretory material, the nucleus moves towards the basal /

the basal part of the cell, and stains more deeply. Due to the accumulation of secretion, the cell membrane at the glandular pole expands greatly and becomes less definite; at the same time the outline of the cell becomes fainter (Pl. II, figs. 4-7). In the final phase, the cell again becomes completely filled with secretory material in the form of vesicles (Pl. II, fig. 8).

The descent of the nucleus and the non-disappearance of the cytoplasmic inclusions, as well as the general lack of mitotic figures, suggests that the cells do not disintegrate, but that they regenerate and again become active. Narrow cells seen among the young cells are probabely in the earliest stage of regeneration. The early secretory phases will be described in the section dealing with the Golgi material.

Golgi apparatus.

The cytoplasm and secretory material does not impregnate deeply with osmic acid as in cells from other glands examined; consequently the cells appear light in colour. Against this background, the silhou-:ette of the impregnated Golgi material presents a contrasting picture which greatly facilitates a detailed study. It would be difficult to imagine greater morphological variations than those displayed by this particular component in the cells of the salivary gland. Morphological changes correlated with the different phases of secretory activity could be followed /

be followed with ease. In describing the Golgi material we shall follow the stages of secretion already outlined. In general, the Golgi material appears as a network frequently described in the somatic cells of vertebrates.

In the dormant cell, with a maximum accumulation of secretory material, the Golgi material is very small, often difficult to impregnate, and consists of a few threads with small thickenings. It lies quite close to the basal membrane and the nucleus. Some of the threads follow the protoplasmic partitions between the secretory vesicles. In outline it forms a basket-like structure on which rests the secretory mass (Pl. I, fig. 3). Parallel with the regeneration of the cell the Golgi substance increases in amount. At the time when the cell gradually enlarges it shows a distinct tendency to spread over a large area always situated above the nucleus (Pl. I, figs. 2,4 and 5). At the time of the maximum expansion of the cell the Golgi material is in the form of a strong network of filaments, lamellar spirals, branched threads and various short rods and granules (Pl. I, fig.8).

When the secretory material accumulates at the pole of the cell next to the lumen, the Golgi material gradually moves to the basal part of the cell, diminishes in quality and in its power to reduce osmic acid as compared with the period of its greatest expansion. Finally it returns to the barely perceptible form with which this description started.

Using /

Using various techniques and times to obtain different intensity of Golgi impregnation, the following phases were observed particularly clearly in material treated by the Mann-Kopsch or modified Ludford technique. In the hypertrophied Golgi net of the regenerated cell (Pl. I, fig. 8), numerous small granules are embedded on lighter threads which are often only faintly outlined. These are the primary secretory granules; later they increase slightly in size. Some of the larger secretory granules, situated as a rule on thickenings of the Golgi threads, have a vesicular form and do not impregnate strongly with osmic acid and do not stain with any of the dyes used (Pl. I, figs. 4,5 and 8). Transition of these vesicles, which still reduce osmic acid, into the vesicles of secretion which no longer reduce osmic acid was not observed. The contents of these larger secretory vesicles appear to be more liquid in nature and gain an affinity for the dyes used for the demonstration of mucus. The larger secretory vesicles are surrounded by ramifications of the Golgi network which sprawls on their surface and separates them into clusters with grape-like structure (Pl. I, fig. 5). With the accumulation of more material these vesicles tend to fuse together, disrupting the outer links of the surrounding Golgi net and expanding to the glandular pole of the cell. The disrupted Golgi net is constri-
:cted towards the basal and lateral cell boundaries and thus /

and thus bring the network of the adjacent cells quite close together, producing an optical illusion that the networks of neighbouring cells anastomose with one another (Pl. I, fig. 1).

Mitochondria.

The mitochondria of regenerated cells which have not yet become active are present as numerous rods, long filaments and as granules. The granules are most numerous in the basal subnuclear part of the cell; rods predominate in other parts of the cytoplasm, and are more or less evenly distributed with a slight concentration on the cell periphery (Pl. II. fig. 1). Bleb-like terminal swellings were sometimes seen, but in an insufficient number to permit any definite conclusions to be drawn. Short rods and granules are more numerous in material fixed in osmic acid containing fixatives. Rods and filaments are arranged parallel to the long axis of the cell. In older cells, under the pressure influenced by the secretory material, the mitochondria retreat systematically to the cytoplasm in the basal and peripheral regions, a few however may be scattered in the cytoplasm between the vesicular areas (Pl. II. figs. 5-8). With the onset of secretory activity, the mitochondria are larger and the majority are in the form of long rods (Pl. II, figs. 1-4). Later, when the secretion is formed and occupies nearly the whole of the cell, the mitochondria are smaller and granules and short rods become more numerous /

numerous (Pl. II, figs. 5-8). Although a large amount of material was used in the course of these experiments, it is difficult to estimate whether the total number of mitochondria decreases, as many small forms, situated between the mucous masses, may easily be overlooked.

Although a few mitochondria may be present at the periphery of the mass of secretion, there is no evidence that they give rise to secretory material. Judging from their changes and presence during all the secre-:tory phases, it is probable that they, as well as the Golgi material, are actively concerned with the sepa-ration from cytoplasm and synthesis of substances utilized in the formation of the secretion.

D. Discussion.

The absence of replacement cells, and mitotic figures amongst young cells, indicates that complete degeneration and replacement of the gland cells by the new ones arising by cell division must be of quite infrequent occurence. Each cell must regenerate after more than one secretory cycle. The strong hypertrophy of the Golgi material in the early phases of secretion; the presence of numerous granules, which enlarge and become vesicular in close contact with the Golgi threads, and the appearance of the demonstrable vesicles of secretory material surrounded by the Golgi network, are all presumptive evidence that the Golgi material is associated /

is associated with the origin of the secretory material. The absence of free secretory granules in the mucus-secreting type of gland cell is not unique. Nassonov (1923) and Bowen (1926) have pointed out that mucous granules seem to be synthesized more rapidly than other secretory material, and that the freeing of the secretory granules from the Golgi threads does not always take place. It is possible that if more violent stimulants were used for inducing secretory activity and evacuation, free granules of secretion which reduce osmic acid might be obtained, but in normal physiologi-:cal conditions, using food as a stimulant, it does not appear to take place.

The mitochondria do not show such a close associa-:tion with the formation of the secretory material, but their arrangement on the surface of the mucous vesicles, intermingled with the reticular Golgi net, suggests that a revision of Cowdry's (1926) conception is desirable. Cowdry suggested that the Golgi apparatus together with the mitochondria may act as membranes where transformation of cytoplasmic materials into secretory products take place. Accepting the above conception we observe in the case of these particular cells an extreme example where a considerable amount of cytoplasm is used up in the production of secretion.

PLATE I.

Drawings of cells of the salivary glands; showing the Golgi material.

All figures from Mann-Kopsch or Kolatchev preparations.

Fig. 1.- Showing the lamellar arrangement of the cells.

Fig. 2.- To show different forms of the Golgi material in neighbouring cells.

Fig. 3.- Cell totally filled with secretion; the Golgi material is very small.

Figs. 4 and 5.- Cells to show that secretory granules become visible in the Golgi zone.

Figs. 6 and 7.- Cross-sections of cells; showing the Golgi material.

Fig. 8.- Regenerated cells with hypertrophied Golgi material.

PLATE I.

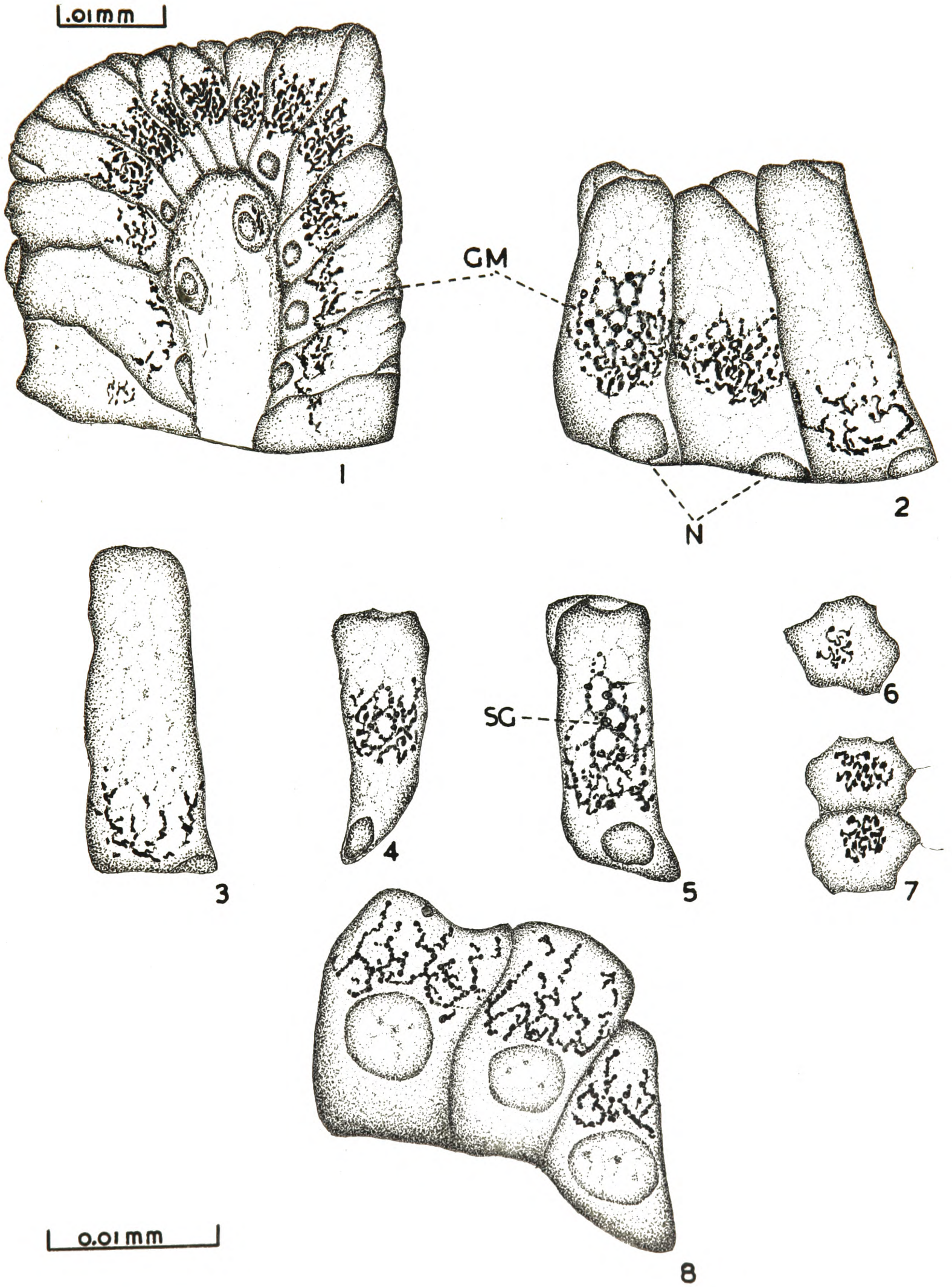


PLATE II.

Drawings of cells of the salivary glands; showing the mitochondria.

All figures from Champy-Kull or Regaud preparations.

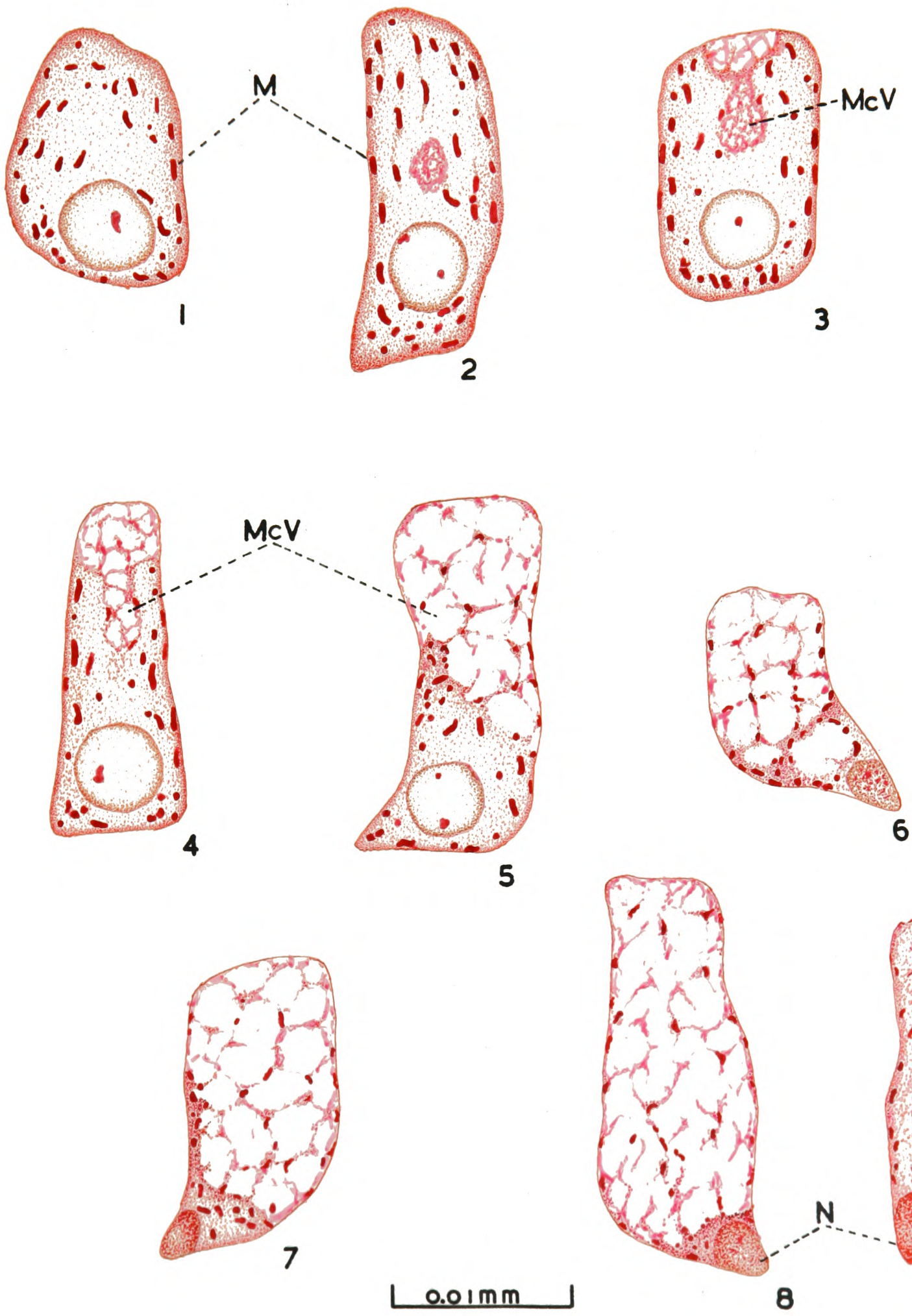
Fig. 1.- Regenerated cell.

Figs. 2 and 3.- Cells to show that the mucous vesicles are formed in the Golgi field.

Figs. 4-8.- Cells to show the gradual accumulation of secretion and its expansion towards the basal part of the cell.

Fig. 9.- Cell after the discharge of secretion.

PLATE II.



6. PANCREAS.

A. Historical.

Since the first students of cytology began to study the activities of the cell, the pancreas has been one of the most popular organ for investigations. Altmann (1890) described the mitochondria of the pancreas as "vegetativen Fäden", and ascribed to them a rôle in the origin of secretion. It was also the pancreas cell in which Holmgren (1902) described his trophospongium. The large size of the cells and the ease with which any cytological technique may be applied were the main reasons which made them attractive objects for study. More varied theories were formulated, and observations made, on the cells of the pancreas than on those of any other vertebrate gland. Some of the earlier workers believed that the mitochondria were the sole participants in the secretory processes, and that by simple budding they produced new secretory granules; for example Arnold (1912). Ma (1924) stated that during inanition in the guinea pig, the fat globules were formed in the interior of the mitochondria of the pancreas. In his later work on the pancreas he formulated a new conception of secretion, and stated, "that in the process of secretion the mitochondrial substance dissociates freeing or unmasking lipoid and giving rise to zymogen". Saguchi (1919), in his studies on the pancreas in the frog, observed that the /

that the elaboration of zymogen granules takes place in the clear area above the nucleus described as the "secretogen area". This author believed that the mitochondria first converge towards this area, and that some of them pass into it and disintegrate to form zymogen granules. Saguchi, however, did not follow the direct transition stages between the mitochondria and the zymogen granules. Other workers negated any idea of mitochondria playing a rôle in secretion (Mislawski, 1912, Orima, 1926).

Following the works of Nassonov (1923) and Bowen (1926), in which the pancreas was one of the glands studied, many workers turned to the Golgi material as a source of secretory materials. Later new methods of vital and supravital staining added support to the belief that the Golgi material played an important part in the origin of secretion (Ludford, 1928, Duthie, 1934,). Many authors,however, disagreed with Nassonov's theory that the Golgi substance is the only cytoplasmic component concerned with the origin of secretory granules (Morelle, 1924). Morelle stated that the granules first arise in connection with the mitochondria and that their final metamor-:phosis is in connection with the Golgi material. This suggestion, purely hypothetical at that time, gained a strong and sound basis after the work of Covell (1928), who was the first to successfully observe living pancreatic cells of the mouse under the microscope. /

the microscope. Covell followed the evacuation of the zymogenic granules after the administration of pilocar-:pine, and the growth of new granules soon after the old ones were extruded into the lumen of the acinus. Covell's method was profitably used by Hirsch (1931-1932), who stimulated the pancreatic cells of the mouse with pilocarpine and kept a single living cell under continuous observation for 30 hours. As the result of these investigations it was definitely concluded that both the mitochondria and the Golgi material participate in secretion. At least four phases in the history of a single granule were observed. Very small granules first appear on the surface of mitochondria in the basal part of the cell. These granules leave the mitochondria and make their way to the Golgi zone, where further growth is continued in association with the Golgi substance. After further growth and changes in staining reactions they leave the Golgi field and proceed towards the glandular pole of the cell. Ultimately they are discharged into the lumen of the acinus. These observations were later substantiated by Duthie (1934).

B. Methods.

In spite of the great ease with which good cytological preparations were obtained, it must be noted that the pancreas is difficult to study when food is used to stimulate the secretory processes.

All methods /

All methods for the demonstration of the mitochondria and the Golgi material, except the silver methods, gave good and constant results. Material fixed in fixatives containing osmium tetroxide is necessary for the study of secretory granules. Contrary to its action on the other organs studied during the present investi-:gations, Regaud's fixative gave uneven fixation of the superficial and deeper parts of the tissue. This uneveness became apparent on staining the sections. As the deeper parts of the tissue need much longer differentiation it is impossible to get an even staining of the superficial and deep layers of cell in the same section, unless very small pieces of tissue are fixed. Usually the deeper parts remain greatly overstained at the time when the surface layer is sufficiently differen-tiated. This feature was not noted in the osmic fixed material. Material fixed for mitochondria, with osmic acid fixatives, gave a very good triple staining with acid fuchsin-toluidine blue-aurantia.

C. Observations.

The acinar cells only were studied; no attention was paid to the islets of Langerhans.

A certain number of pancreatic cells are associated together to form an acinus with an irregular lumen in the centre. The cells are of an irregular pyramidal shape with the narrow apex bordering the acinar lumen. The large spherical nucleus, depending on the functional stage of the cell, lies near the basal membrane or close /

close to the middle region of the cell. In the normal resting phase subsequent to a 24 hours fast, the secretory (zymogenic) granules crowd the portion of the cell between the nucleus and the lumen of the acinus. They are spherical in shape and of various sizes. The smaller granules are usually deeply stained with acid fuchsin, while the larger ones stain very faintly and are more often coloured a faint yellow by the picric acid used in differentiation. The larger granules are vesicular in form (Pl. I, fig. 6). Very rarely, granules extend towards the basal part of the cell, and in this case they push the nucleus close to the basal membrane (Pl. I, figs. 3 and 7). Soon after feeding (half an hour), a great number of the secretory granules are evacuated, but total evacuation of secretory granules was never observed. In many cells, unstained vacuoles mark the position formerly occupied by secretory granules.

Previous workers have stated, that an acinus acts as an autonomous unit and that all the cells in a single acinus become active synchronously (Hirsch, 1931-32, Ries, 1935). This was confirmed during the present investigation. The number of granules present in an acinus seem to vary greatly. Some of the acini fixed half an hour after feeding contain few granules, while others may contain a large number and remain in an unchanged condition which is similar to the resting phase. It was difficult to follow the order in which the evacuation /

the evacuation of the granules takes place. The release of granules begins soon after feeding and is continued for two hours after the intake of food. Some acini, which had previously discharged their contents, begin a new secretory phase one hour after feeding. Numerous very small granules are visible in the supranuclear zone of these cells (Pl. II, fig. 2). They usually stain very deeply with acid fuchsin, and in this they differ markedly from the rest of the granules. These are, no doubt, the granules which Bensley (1929) regarded as young zymogen granules, and called "prozymogen granules"; they stain with neutral red in the living cell. They were observed during the present work in material stained supravitally with neutral red and also in unstained living cells. In unstained living cells they appear as highly refractile granules. No granules were observed in the subnuclear region of cells in fixed and stained preparations. Two hours after feeding, there is great variation between the different acini. Some are entering upon the secretory phase while others contain large secretory granules situated between the numerous small granules. The process of restitution of new granules progresses gradually, and 5-6 hours after feeding the majority of the cells in all the acini are filled with granules to the same extent as after a 24 hours fast. Three hours after feeding, and later, observations are greatly hindered by the large number of secretory /

of secretory granules present in the cytoplasm.

Golgi apparatus.

During the present investigations the Golgi material was demonstrated in pancreatic cells in every phase of activity, both during a fast when there is a maximal accumulation of secretory granules in the cytoplasm (Pl. I, fig. 3) and at each stage after feeding. The Golgi material always forms a more or less organized body situated at the pole of the nucleus next to the lumen. There were slight changes in its position depending on the functional stage of the cell, so that it may lie close to the acinar lumen or towards the middle region of the cell. Its structure changes according to the stage of secretion. A variety of twisted strips, threads and batonets form a very complicated net impregnated with osmic acid. The net-like structure appears to form a support and framework for a substance which reduces osmium tetroxide slightly more vigorously than the rest of cytoplasm (Pl. I, figs. 1-4 and Pl. II. figs. 1-8). Careful examination shows that osmophilic threads and strips surround and divide the less strongly osmophilic substance into numerous separate regions.

Marked changes were noted in the Golgi material during the production of secretion. After 24 hours fast the Golgi material is a rather compressed body laying in the supranuclear region, and usually partially surrounded by the secretory granules (Pl. I, figs. 1-3). Deeply /

Deeply impregnated threads are very prominent and sharply outlined. The cytoplasm between them, reduces osmium tetroxide moderately. The first visible morphological changes were noticed one hour after feeding, when there is a loosening of the osmophilic threads, and the field covered by the Golgi material is consi-:derably larger than during a fast. Small sharply outlined swellings are seen in the osmophilic links. The cytoplasm which fills the spaces between the osmophilic links reduces osmium tetroxide more strongly than during the inactive phase (Pl. II, fig. 1). Two hours after feeding the osmophilic substance between the threads is even darker. The whole Golgi field is now quite sharply outlined and stands out from the rest of the cytoplasm (Pl. II, figs. 3 and 4). The osmophilic threads are thinner and less definitely outlined, but numerous swellings are situated along them. Three hours after feeding the osmophilic links on the glandular side of the Golgi material appear to lose their continuity and numerous granules, which are still osmophilic, move away from the Golgi field. These are the secretory granules which at this stage are intimately connected with the Golgi material (Pl. II, figs. 5 and 6). The secretory granules decrease in number during the later hours after ingestion, but are present at any time after feeding as well as in birds with constant access to food (Pl. II, figs. 7 and 8). /

(Pl. II, figs. 7 and 8). Five hours after the intake of food the Golgi material becomes more compact than it was 3-4 hours after feeding.

Mitochondria.

The mitochondria of the pancreatic cells were extensively studied; there are several theories as to their rôle in secretion.

The mitochondria of the pancreatic cells of domestic fowl are of the same pattern as that described by most workers on the pancreas of other animals. They are remarkable for their size and length. A great variety of forms are encountered in each cell- long filaments often extending for 2/3 the legth of the cell, short rods, and granules. The filaments are generally slightly tortuous. All of them appear to lie for the most part parallel to the long axis of the cell, but may also lie parallel to the basal membrane (Pl. I, figs. 5 and 6). In the osmic fixed material the presence of secretory granules makes the examination of the mitochondria in the supranuclear zone difficult or impossible. In material fixed and stained according to Regaud's method, they are clearly visible in all parts of the cell (Pl. I, fig. 5). The long filaments are commonly seen along the lateral cell walls. Sometimes swellings were found, by careful study, to be nothing but sections through the tortuous bendings of the long filaments. Some changes were observed in the mitochondria /

the mitochondria after feeding, but they are not so striking as, for example, in the liver cells. At the time of increased cellular activity long tortuous forms are very seldom seen and more regular rods prevail. This is most notable 2-3 hours after feeding. After that time the mitochondrial pattern is the same as in fasting birds.

D. Discussion.

The acinar cells of the pancreas were extensively studied by many cytologists. The majority of workers used stimulants (pilocarpine) which evacuate the secretory granules more thoroughly than is normal. There is always the danger that such stimulants might have a harmful effect upon the cells.

When food is used as a stimulant to bring about the discharge of the secretion of the pancreatic cell, a comparatively small number of secretory granules are evacuated, and observation is therefore rendered much more difficult. In spite of these difficulties, changes were observed during the present work, both in the Golgi material and in the mitochondria. The changes in the latter, however, are not striking. The changes in the Golgi material strongly suggest that it plays an important part in secretory activity. The majority of cytologists, with the exception of a few of the earlier ones, drew attention to the connection between the Golgi material of the pancreatic cells and the origin /

and the origin of the granules of secretion (Nassonov, 1923, Bowen, 1926, Beams, 1930, Bufio, 1931, Hirsch, 1931-32, Ries, 1935, Duthie, 1934). No direct connection was observed between the mitochondria and the granules of secretion, or between the behaviour of the mitochondria and the secretory processes.

PLATE I.

Drawings of the pancreatic cells; showing the Golgi material and mitochondria.

Figs. 1-4 from Kolatchev or Ludford preparations.

Fig. 5. from Regaud preparations.

Figs. 6 and 7 from Meves preparations.

Figs. 1-4.- Cells after 24 hours fasting; showing Golgi material.

Fig. 5.- Cells, showing mitochondria only.

Figs. 6 and 7.- Cells, showing mitochondria and secretory granules.

PLATE I.

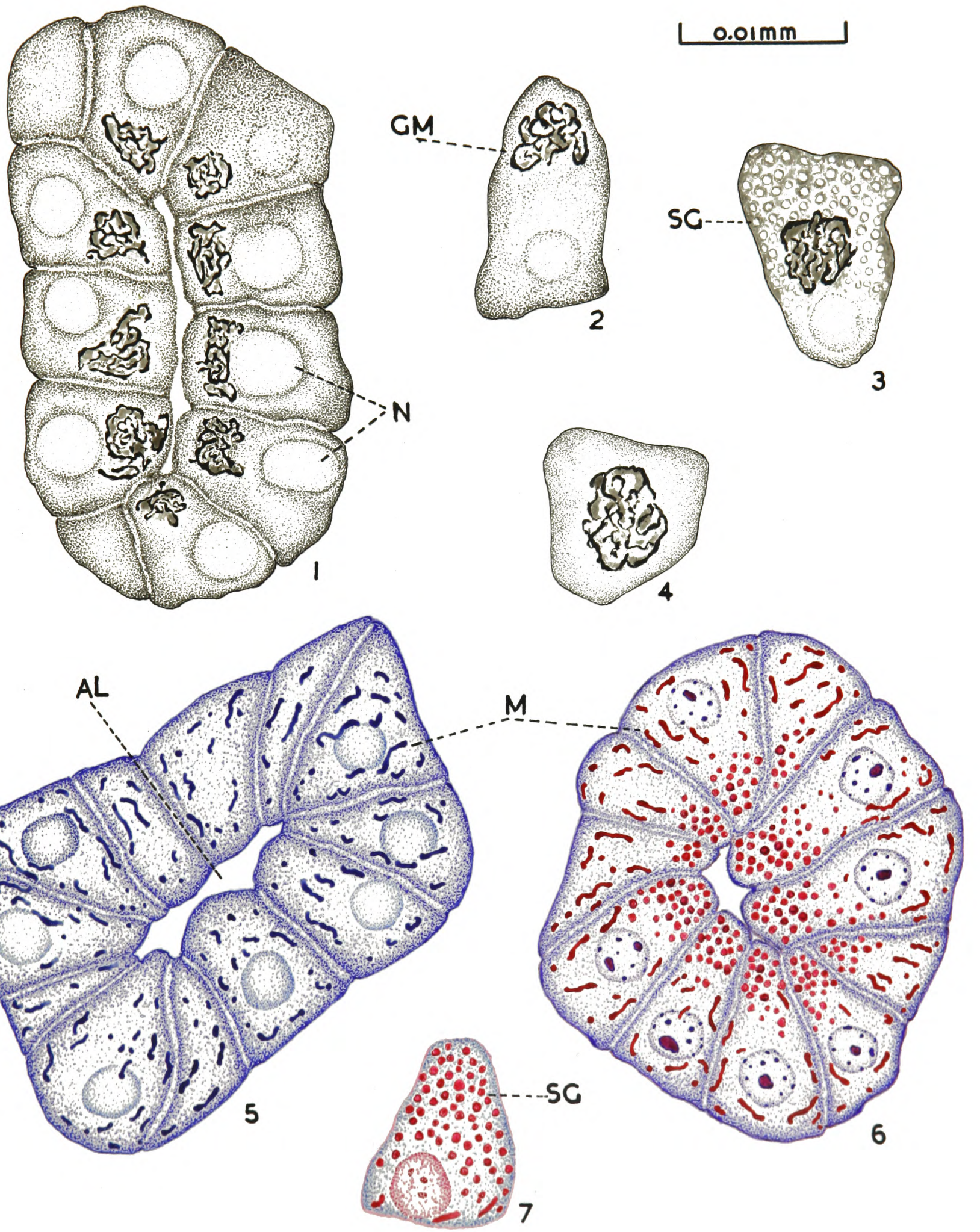


PLATE II.

Drawings of the pancreatic cells; showing the Golgi material.

All figures from Kolatchev or Mann-Kopsch preparations.

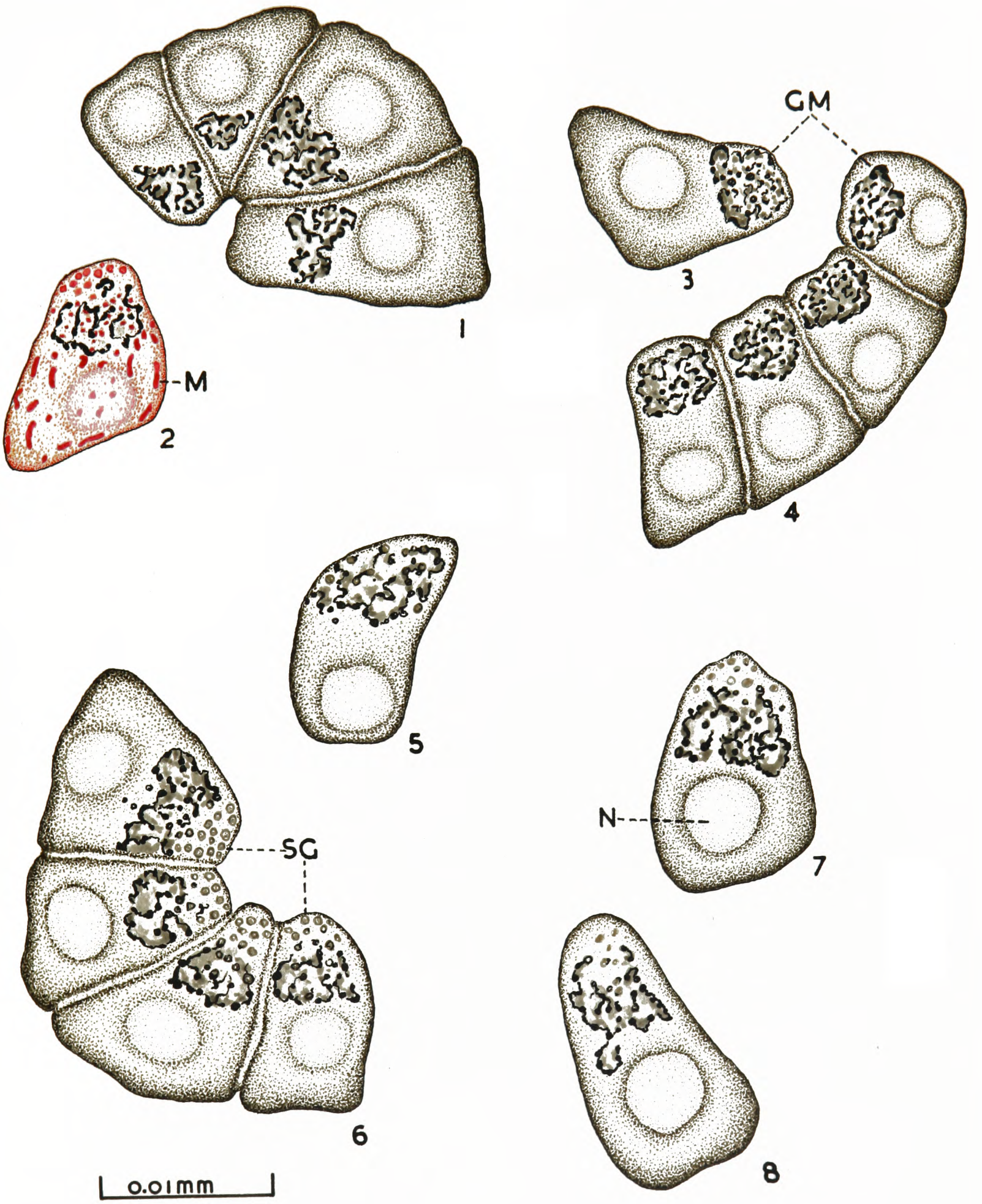
Figs. 1 and 2.- Cells, one hour after feeding.

Figs. 3 and 4.- Cells, two hours after feeding; showing first secretory granules.

Figs. 5 and 6.- Cells three hours after feeding; showing secretory granules leaving Golgi zone.

Figs. 7 and 8.- Cells of the birds with constant access to food.

PLATE II.



7. LIVER.

A. Historical.

The dual rôle played by the liver cells in the glycogen-glucose equilibrium and in bile secretion, and the characteristic lobular structure of the liver, has stimulated much research on the functions of these cells under different physiological conditions. According to the available literature on the cytology of the liver, various aspects of the problem have been studied and many theories have been revised in an attempt to elucide the functions of the hepatic cells. Until the researches of Cramer and Ludford (1927), who successfully impregnated the Golgi material, nothing was known about this component of the liver cell.

The function of the mitochondria in the hepatic lobule was the cause of much speculation on the part of several authors. According to the nature of the changes in the form and disposition of the mitochondria, their function was ascribed either to carbohydrate assimilation or to the secretion of bile. Inconsisten-:cies are prevalent in the literature dealing with the form of the mitochondria of the hepatic cell. Noël and Pallot (1933-34) interpreted the numerous alterations in the morphology of the mitochondria as an expression of activity in connection with the secretion of bile, rather than in response to other functions /

functions of the liver. It is generally accepted that spherical mitochondria represent an inactive phase while the filamentous type represents the functional state. During starvation, the mitochondria of the liver tend to become spherical (Meersseman, 1939, Dalton, 1933, Mc Cudry, 1939-40, Steffens, 1941), but this is denied by Noël and Pallot (1933-34). Other workers induced by various means in different animals, hypo- and hyperglycemic stages and attributed mitochon-:drial changes more or less exclusively to carbohydrate metabolism. In support of this theory they stated, that a tendency towards enspherulation and hypertrophy marks both hypo- and hyperglycemia (Mc A. Kater, 1931, Clark and Hair, 1932, Hall and Mac Kay, 1933, Mc A. Kater, 1931 and 1937). Among other functions attributed to the mitochondria of the liver cells was that fat globules are formed within them (Kater and Smith, 1932, Noël and Pallot, 1933-34).

All the earlier workers mention the great technical difficulties involved in demonstrating the Golgi material in hepatic cells. Accurate observations on the Golgi apparatus in the liver were only made comparatively recently (since Cramer and Ludford's publication in 1927). Works based on silver impregnation (Dornesco, 1929-31, Imai, 1935, Pfuhl and Dienstbach, 1938) must be accepted very sceptically as most reliable observations have shown that silver nitrate methods /

nitrate methods fail to impregnate liver cells successfully (Subramaniam, 1938, Cramer and Ludford, 1926-27, Dalton, 1933, Pollister, 1932). Most investigations have shown that changes take place in the morphology of the Golgi material during increased cell activity (Cramer and Ludford, 1926-27, Ludford, 1928, Pollister, 1932, Dalton, 1933, Subramaniam, 1938). Cramer and Ludford (1926-27) and Subramaniam (1938), as the result of their study of the Golgi apparatus in the liver cell, concluded that the constituents of the bile were formed in relationship with the Golgi material which, in the process of secretion, enlarges considerably.

Kater (1933 and 1937) came to the conclusion that "mitochondrial pattern of the chicken liver is so irregular that nothing can be regarded as normal". An interesting ontogenetic observation on the cytology of the liver of the chick was made by Dalton (1933), who gave a thorough description of changes in the cytoplasmic components from the earliest embryonic development to the time of hatching. He also described changes after hatching which were caused by fasting and feeding. Dalton concluded that there is an increase both in the number and length of the mitochon-:dria in the early period of development (3-11 days). From the 11-th to 19-th day of incubation, there is an increase in the size and number of the mitochondria correlated with a rapid increase in the amount of glycogen /

glycogen and fat appearing in the hepatic cell. From the 19-th day to the time of hatching there is a decrease in length and an increase in the number of the mitochondria. If no food is given during the first 5 days after hatching, yolk is used as a source of energy and the mitochondrial pattern does not change. Chicks 5 days after hatching were found to show a definite correlation between feeding and the length and number of the mitochondria. Long mitochondria are indicative of heightened activity, while fasting brings about a shortening and also an increase in the number of the mitochondria. Dalton did not find any intralobular variations. From the 7-th up to 14-th day the Golgi network enlarges, but after this time there is no detectable change during the incubation period. A second change occurs after hatching and is induced in the liver cell by the increased activity which follows feeding. In the liver of fasting chickens the Golgi material is always extensive and is located close to the bile capillary. Dalton concluded that no evidence of a morphological character has been present which would indicate a specific action of the mitochondria in synthesis, or the Golgi material in secretion. The mitochondria and Golgi apparatus, however, show a characteristic pattern both with increased and decreased cellular activity. During the present investigations, which might be described as a continuation /

a continuation and extension of Dalton's work, some further interesting results were obtained on the behaviour of cytoplasmic components of liver cells during fasting and at regular intervals after feeding.

B. Methods.

The liver appears to be the most difficult of the tissues used during the present investigations, as regards both penetration and fixation especially with fluids containing osmium tetroxide. As stated by many previous workers, osmic fixatives were found to fix only the very narrow and superficial layer of the tissue. Meves' fixative was the best of this group, and was used chiefly in the study of the secretory granules, but it never penetrated deeper than a few rows of cells. The deeper parts of the tissue are in general unsuitable for comparative studies, as the mitochondria as well as the secretory granules swell greatly and appear as large vesicles of various sizes. Material treated by Regaud's method and stained with Regaud's haematoxylin or with Bensley's acid fuchsin light green was the most suitable, and was used as a basic method for all comparative studies. As found by previous investigators (Cramer and Ludford, and Subramaniam) the silver nitrate methods fail completely to demonstrate the Golgi material. Ludford's method proved to be best and most constant. Mann-Kopsch and Kolatchev /

and Kolatchev may be used, but after impregnation the osmic acid must be reduced in distilled water at 37°C for at least two days. Without this modification no successful blackening of the Golgi material was obtained at any time. Bleaching is often needed as the cytoplasm tends to impregnate a deep brown colour.

C. Observations.

Unlike the liver of mammals, that of the domestic fowl is much simpler and has no true lobular structure. The liver epithelium surrounds the tubules which are the intercellular bile canaliculi. The hepatic cell is an irregular polyhedral pyramid, with its apex bordering on the lumen of the bile canaliculus and the basal part in contact with the blood capillaries. The large spherical nucleus lies in the basal part of the cell, and in the surrounding cytoplasm a few globules of fat are usually blackened in material fixed by Meves' method and stained for fat. All preparations for the demonstration of the mitochondria and the Golgi material were examined carefully, but no inter-:lobular variations were noticed.

Golgi apparatus.

Comparing material fixed and impregnated after a 24 hours fast with material taken from birds killed at hourly intervals after feeding, some rather interes-:ting observations were made. The examination of these preparations /

preparations indicated that the Golgi material plays a specific part in secretion. In the hepatic cells of fasting chicks, the Golgi material is located close to the nucleus. It consists of somewhat thick, sausage-like rods, with a few links between them. The thick rods are loosely placed in the form of an umbrella above the nucleus (Pl. I, figs. 1-5). The rods do not show much detail in their structure and are fairly uniform in thickness. The first changes are perceptible one hour after the intake of food. Thick Golgi rods elongate slightly and become arranged, as the rays in an umbrella, above the nucleus; this feature is striking. Cross-links are not seen. As they elongate, the Golgi rods become thinner (Pl. II, fig. 1). Marked changes in the Golgi material become visible two hours after a meal. Long rods become twisted and connected with links, thus forming a more complicated structure containing many cross-links. Thickenings and a few granules were observed in the course of the Golgi links, and were blackened as deeply as the links (Pl. II, fig. 2). Four hours after feeding, the Golgi material spreads out widely and numerous small granules, arranged in rows, replace many of the links. It appears often as though the thick links are split into thinner threads with a great number of granules intimately connected with them. Most of the granules at this time are small and deeply blackened while the threads are /

threads are much fainter in outline (Pl. II. figs. 3 and 4). Six hours after feeding, some granules are seen beyond the Golgi material in the form of large vesicles very faintly outlined in osmic preparations. The thick links are replaced by thinner threads, or rather grey lamellae, which spread between the small dark granules. The cytoplasm between the Golgi links and granules takes on a darker colour and appears to outline the whole Golgi field (Pl. II, figs. 5 and 6). In the hepatic cells of birds which had constant access to food without a previous fast, the Golgi material is a strong umbrella shaped structure with few thickenings and sometimes with a few granules between and along the thick links and rods composing the Golgi material (Pl. II, fig. 7).

As many as six hours after the intake of food are needed to cover the whole secretory cycle in the liver cell, and to observe the characteristic morphological changes in the Golgi material during increased cellular activity. The first secretory granules appear in intimate association with the Golgi material and are impregnated as deeply as the Golgi links. In the later stages, numerous granules are present and the Golgi lamellae link them together to form a structure which is best described as the Golgi body. These observations conform in nearly all respects with Subramaniam's studies on the Golgi apparatus in the liver of Rhacophorus maculatus Gray (1938)./

Rhacophorus maculatus Gray (1938).

Mitochondria.

The morphological changes of the mitochondria in connection with the various functional phases of the hepatic cells induced by fasting and feeding were considered during this investigation. No reference is made to the glycogen-glucose equilibrium or to the secretion of bile. More striking changes in the morphology of the mitochondria of the hepatic cells were observed during increased cellular activity than in any other of the gland cells investigated. After a 24 hours fast the mitochondria are in the form of granules, ovoid bodies, and short rods. Granules and ovoid forms are most numerous and are present exclusi-:vely in the basal part of cells. Rods intermingled with granules are seen in the supranuclear zone. The supranuclear mitochondria tend to be arranged more or less parallel to the long axis of the cell, while those in the subnuclear region are scattered at random in the cytoplasm (Pl. I, fig. 7). A strikingly different mitochondrial pattern from that seen during a fast becomes visible one hour after feeding, and is very prominent after two hours. A few granules only are present while rods and filaments are the predominant forms. The mitochondria appear to be thinner, and to be slightly wavy; they have a typical polar orientation from the basal to the glandular pole. They are more or less equally distributed throughout the cytoplasm and appear /

and appear to be more numerous than during the fasting condition (Pl. I. fig. 6). Some filaments show deeper stained segments or spherical bodies. No further change in the shape or polar arrangement of the mito-:chondria was observed in material fixed three hours after a meal, but in many cells a light irregular area free of mitochondria was present around the nucleus. The mitochondria appear to be pushed aside and to accumulate on the border of these irregular areas, thus giving a fenestrated appearance to the cytoplasm around the nucleus.

Very few changes in the morphology of the mito-:chondria were noticed in the later phases of digestion. In material fixed by Meves' method, 4-5 hours after feeding numerous very small secretory granules as well as a few larger granules were present near the glandular pole of the cell. The mitochondria of the hepatic cells of birds with constant access to food show little difference in form to those present two hours after the intake of food. Filaments are predominant, but rods appear to be more numerous than in the early phases of digestion.

This investigation shows that the mitochondria, like the Golgi material, undergo marked changes during increased secretory activity of the hepatic cells. This is in close agreement with the observations of many previous workers who stated that granular mito-:chondria in these cells represent an inactive stage while /

while filamentous forms denote increased activity.

(a) Dark cells.

Many histologists and cytologists observed that certain liver cells take on a deeper stain than their neighbours and possess a greater ability to reduce osmium tetroxide. The depth of staining observed varies from a light, barely noticeable shade, to an almost black colour which excludes any study of the cytoplasm. The number of these cells varies. This type of cell was present in sections examined during the present investigation. They vary in number, but the variations do not appear to be connected with the phases of secretory activity. Very little is known about the nature of the intercellular changes which cause these cells to stain in a different manner to their neighbours. Some workers regard them as dege-:nerating cells (Roeckel, 1938, Weatherford, 1935), while others state that well fixed material shows that they are perfectly normal cells which vary only in their staining properties (Dalton, 1933).

D. Discussion.

In spite of the difficulties encountered by various workers, especially in obtaining good Golgi preparations, and in spite of the accepted opinion that the liver is a difficult and complicated gland for cytological study; very spectacular morphological changes both of the Golgi material and the mitochondria were observed /

were observed during the present investigation. There appears to be no support for the conclusion drawn by Kater (1933-37), that the mitochondria show no regularity in the liver of domestic fowl as regards their morphological features, and that the mitochondrial pattern is so irregular that it can never be regarded as normal. Kater's results can only be attributed to the harmful effects of the stimulants used or to the complicated methods of feeding which he employed. Steffens (1941) appears to support this view. He states that feeding a single pure diet leads to deficiencies in the other essentials of a balanced diet and hence would alter the normal metabolism.

The liver of the domestic fowl does not possess a lobular structure, and no interlobular differences, similar to those of the mammals' liver, were encountered; this agrees with Dalton's work (1933). All changes observed in the cytoplasmic components of the liver cell are closely connected with cellular activity and secretory phenomena. The very slow morphological changes observed in the Golgi material, render the hepatic cells of the fowl ideal material for the study of the phases of secretion. It is very evident that the granules of secretion appear in close topo-:graphical relationship to the Golgi material, and that the Golgi material undergoes changes of form and disposition which are correlated with the stages of secretory activity. The morphological alterations of the mitochondria /

of the mitochondria are no less closely connected with the increase of cellular activity. One hour after feeding the filamentous mitochondria become oriented parallel to the main axis of the cell; this orientation is much more striking than any which occurs in the other glandular epithelia investigated during the present work. Whatever the function of the mitochondria may be, their rhythm depends entirely on the time of feeding and the response appears to be much quicker than that observed in other animals. It is the generally accepted opinion that granular mitochondria occur during the inactive stage, that the presence of filaments indicate functional activity, and that the morphology of the mitochondria expresses alterations in the cytoplasmic processes.

PLATE I.

Drawings of the liver cells; showing the Golgi material and mitochondria.

Figs. 1-5 from Ludford preparations.

Figs. 6 and 7 from Regaud preparations.

Figs. 1-5.- Cells of the birds killed after 24 hours fasting; showing Golgi material.

Fig. 6.- Cells of the birds killed two hours after feeding; showing marked polar orientation of mitochondria.

Fig. 7.- Cells of the birds killed after 24 hours fasting; granular mitochondria are predominant.

PLATE I.

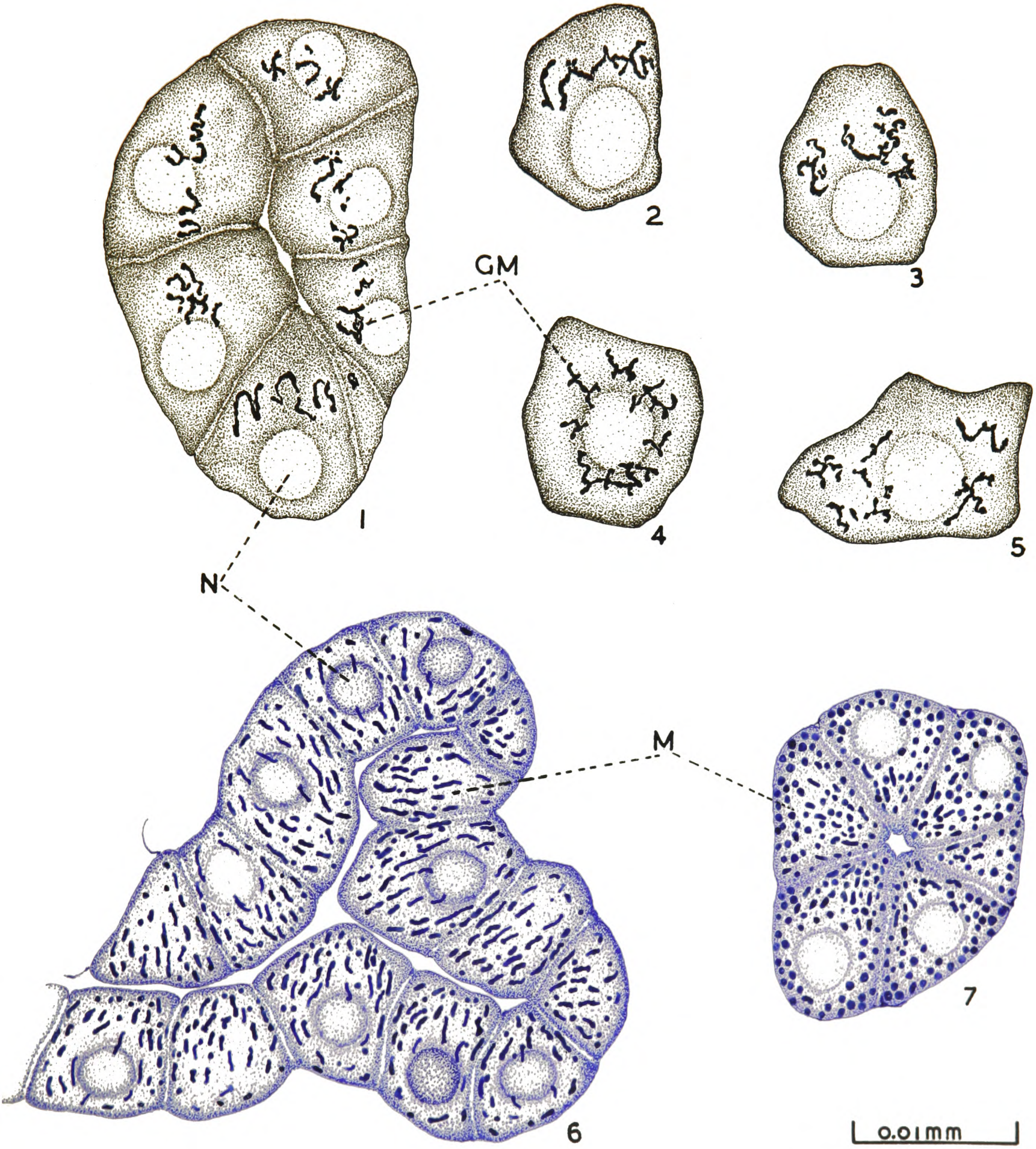


PLATE II.

Drawings of the liver cells, of the birds killed after feeding; showing the Golgi material.

All figures from Ludford preparations.

Fig. 1.- Cells, one hour after feeding.

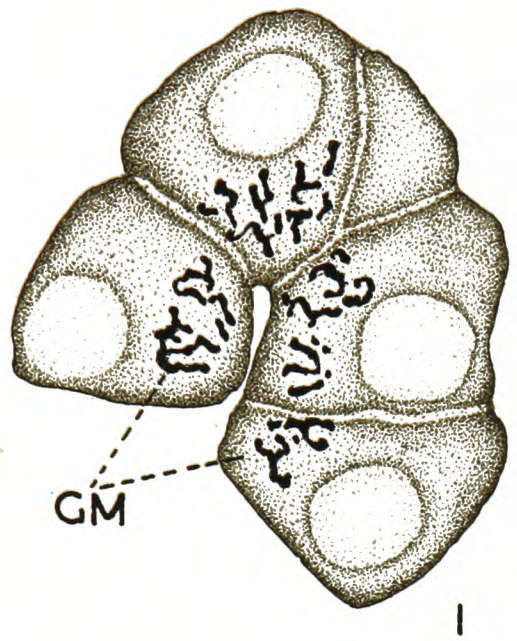
Fig. 2.- Cells, 2-3 hours after feeding.

Figs. 3 and 4.- Cells four hours after feeding; showing numerous small secretory granules.

Figs. 5 and 6.- Cells, six hours after feeding; large secretory granules are seen in Golgi field.

Fig. 7.- Cells of the birds with constant access to food.

PLATE II.



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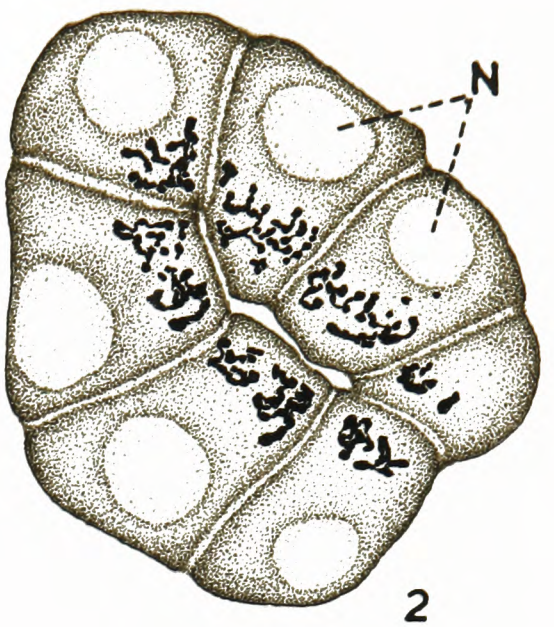

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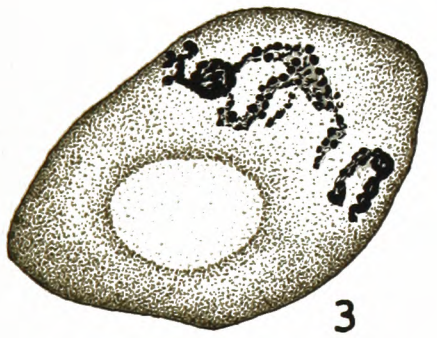

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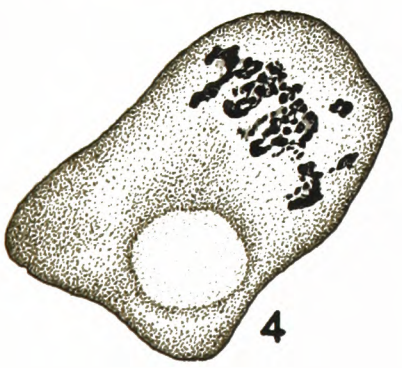

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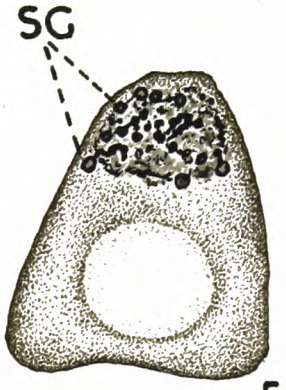

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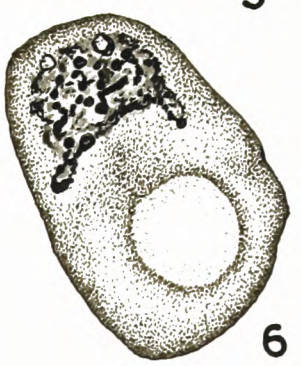

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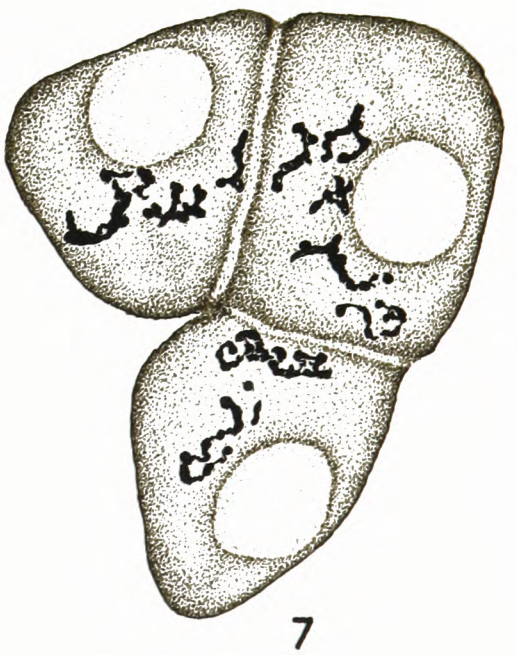

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V. GENERAL DISCUSSION.

The function of the glandular cell is the elaboration of active substances described as " secretion". The elaboration of these substances in the cytoplasm follows a definite order of events and, at a certain phase in the cycle, results in the formation of granules, which are the products of the activity of the cytoplasm. During this complicated process the different cytoplasmic components of all gland cells undergo morphological changes which are correlated with the phases of the secretory cycle. As the visible changes in the extra nuclear cellular components are the principal, if not the sole, key to the understanding of cellular activity, they have become one of the main objects of cytological study. Each cytoplasmic component has its own specific rôle to play in the secretory process. Many of the earlier theories are now discarded and new ones have been advanced. With improvements in cytological technique new information has been gathered from time to time, but new problems have also arisen.

The Golgi material and mitochondria are two important cytoplasmic components of gland cells which have been studied by many cytologists. With few exceptions, the Golgi material cannot be seen in the living cell, and in the past this has caused considerable controversy as to its reality. It is now unanimously agreed that the Golgi material is a definite cell component /

component and that it takes part in the elaboration of secretory granules, but there is still disagreement as to its structure, and the rôle which it plays during secretion. The earlier observations were based chiefly on osmic acid and silver methods, and the majority of cytologists believed that the Golgi material of glandular cells was in the form of a network (Nassonov, 1923-26, Bowen, 1923-29, Ludford, 1930-31, Cramer and Ludford, 1925). In recent years many investigators have stated that this conception is erroneous, and students of vital staining have refuted the existence of the classical Golgi network in the living cell. Parat and Painlevé (1925), as the result of their observations on the pancreas of fishes and amphibia, concluded that the Golgi apparatus in the living cell is in reality composed of a group of vesicles, the walls of which, upon fixation, become impregnated with osmic acid. This belief was supported by plant cytologists (Dangeard and Guilliermond, 1928) who demonstrated the presence of the "vacuome" in the plant cells. The conception has quite recently been revised by Baker (1944). He believes that the Golgi material consists of vacuoles with fluid content which are surrounded by, or in contact with, lipoidal substances. Baker, therefore, concluded that many of the classical Golgi figures are artifacts caused by fixation and shrinkage of the vacuoles which are in reality part of the living Golgi structure.

The behaviour /

The behaviour of the Golgi material, under different physiological conditions in the same type of cell, is conclusive proof that it is not an artifact, but a real component of the cytoplasm. The work of Nassonov and of Bowen was the turning point from which cytologists began to look upon the Golgi material as the centre from which the secretory processes take place. A definite correlation between the form of the Golgi material and the functional activity of the cell has been noted by most cytologists (Nassonov, 1923, Bowen, 1925-26, Jacobs, 1925, Cowdry, 1924, Corti, 1926, Subramaniam, 1938), and was observed in the course of the present work. The final elaboration of secretory granules is generally visible within the Golgi field; this was confirmed by the present writer. The observa-:tion of Hirsch (1931-32) on the living cells of the pancreas, and work with tissues stained by vital and supravital methods, has resulted in a tendency to ascribe to the Golgi material the rôle of a condensation membrane. It is well known that certain dyes, such as trypan blue and neutral red, when injected into an animal are electively condensed into droplets in that part of the cytoplasm where the Golgi material of the cells of the liver, kidney and other tissues is situated. Another notable theory is that of Subramaniam (1938) who believes that the Golgi material secretes intracellular enzymes which in turn are responsible for the synthesis of secretory material.

Mitochondria /

Mitochondria are invariably present in all glandular cells. They undergo morphological changes during secretory phenomena which are, as a rule, less notable than those of the Golgi material, but nevertheless there is evidence that they play an important part in the formation of secretion. Various theories, of which many were never widely accepted, were put forward by different workers. Many of them were purely hypothetical and were not supported by morphological observations. Most of the theories accepted at the present time claim, in one way or other, that mitochondria are the organoids of elective functions in the cell. All these conceptions, with their various modifications, date from Regaud's "electosome theory" (1910). According to this theory, mitochondria absorb from the surrounding cytoplasm the substances essential to the formation of secretion. This theory became more realistic and gained much support with the latest developments and improvements in cytological technique. The selective staining of mitochondria in a living cell with certain dyes (Janus green B) is actually the elective condensation and accumulation of the dye in these cell components. The work of Hirsch (1931-32) and Duthie (1934) supports this theory. They found that in living pancreatic cells the secretory granules first become visible (at least in these cells), in close association with the mitochondria. Further information regarding the rôle of the mitochondria was supplied by plant /

by plant cytologists. During the differentiation of the cells of green plants, some of the mitochondria are transformed into chloroplasts, while the remainder are unchanged (Guilliermond, 1941). It is known that the chloroplasts which originate from the mitochondria are the substratum of chlorophyll.

The present work was undertaken in order to study, during different funtional phases, the morpholo-:gical changes of the cytoplasmic components of the various gland cells of the alimentary tract and associated glands of the fowl.

It was determined that a 24 hours fast brings about a phase of complete inactivity in practically all the gland cells investigated, and that feeding stimulates the cells to enter upon a period of secretory activity. It was observed that the Golgi material and mitochondria undergo changes of structure and dispo-:sition, and that these changes are correlated with the phases of activity of the cell. The nature of the morphological changes, their intensity, and the rate at which they progress, varies greatly in different cells, and is closely connected with a specific rhythm characteristic of each particular group of cells. While the mucus secreting cells, both of the salivary glands and the goblet cells of the intestinal lining, appear to be more autonomous in their action and less capable of being influenced directly by the intake of food than by some other heterological factors, most of the other /

of the other groups of the cells demonstrate a close correlation between intracellular changes and the digestion of food.

The rhythm of the secretory process, and the morphological changes, connected with it varies greatly not only between cells of different glands, but between particular cells of the same gland. Some of the gland cells such as the gastric and pancreatic cells, appear to exhibit a more or less constant secretory balance, with variations depending on the intake of food and periods of fasting; they do not appear to suppress their activities after a short fast. Their reaction to the intake of food appears to be less marked than that of other gland cells. Other cells, including those of the intestinal epithelium which are easily brought to the resting stage by short period of fasting, become strongly sensitized to the subsequent administration of food and show profound morphological changes immediately upon direct contact with the food. A gradual decline in response, and in the morphological changes of the cytoplasmic components, of cells in the posterior part of the alimentary tract suggests that this part performs a different task and is engaged almost exclusively in taking up the products of digestion from the lumen, while the cells of the anterior part are chiefly secretory in fuction.

A great variety of cells were examined during the course of the present study. Judging only from their different /

different affinities for stains and properties of reducing osmium tetroxide, the products of their action are radically different. Nevertheless there appears to be no fundamental difference in the mode of their synthetic operations. Although the final product of activity varies in different types of cell, certain constant morphological changes always accompany its production. The final stages in the formation of secretory granules always take place in the Golgi field. Whatever the structure of the Golgi material in the living cell may be, fixed material always reveals marked morphological changes in the same type of cell under different physiological conditions. The very rapid and profound quantitative increase in the Golgi material of the epithelial cells of the upper part of the intestinal canal, and the qualitative increase in its power of reducing osmium tetroxide, confirm conclusively the belief of many previous workers that the Golgi material is an organelle which actively participates in secretory phenomena. The variability of form, and the changes observed in the Golgi material during different physiological phases in the same type of cells, strongly supports the view first expressed by Bowen (1926), and accepted by many recent cytologists including Gresson (personal communication), that the Golgi material is not a structure with permanent form, but is a specific substance which appears in different forms depending on the functional /

on the functional stage of the cell.

In the course of the present observations on the mitochondria, it was noticed that the behaviour of these cell components varies in different types of cell. All the changes observed, which were least marked in the pancreas and were most striking in the liver cell, could not be accepted as proof of the direct participation of the mitochondria in the secretory phenomena. Of the morphological changes undergone by the mitochondria, the most notable are those concerned with their polar orientation parallel to the long axis of the cell. More regular, elongated forms with a marked polar orientation were observed by the writer in all gland cells during the active phase. This agrees with the work of Pollister (1941). He held that the thread-like mitochondria are arranged parallel with the direct course of diffusion through the cytoplasm, and are influenced by the parallel orienta-:tion of the long protein molecules. It is highly probable that the morphology, and especially the arrangement of these cell components, is more the reflection of the activities taking place within the cytoplasm than an indication of their direct partici-:pation in the secretory process.

VI. SUMMARY.

Proventriculus.

1. The Golgi material of the cells of the surface epithelium, the neck cells and mucous neck cells, is situated above the glandular pole of the nucleus. In the zymogenic cells it always lies on the level of the bottom of the intercellular clefts. In the zymogenic cells the apparent reversal of polarity of the Golgi material is due to the movement of the nucleus.

2. The mitochondria of the surface epithelium and the mucous neck cells are very delicate filaments. In the zymogenic cells, thick rods and granules are usual. The functional stage is marked by the presence of long filaments and a marked polarity of the mitochondria.

3. Secretory granules arise in close connection with the Golgi material. All the gastric cells are in a constant balance of secretory activity. Feeding accelerates the evacuation of secretion and immediately stimulates new production. A total expulsion of secretory granules never takes place in any of the gastric cells after feeding.

Gizzard.

1. The cells of the gizzard differ from other gland cells. The keratinoid material is the secretory product. The Golgi material in the gizzard cell does not show /

not show any changes after feeding. The secretory process is very slow and is independent of the diges-:tion of food. Secretion leads finally to the degeneration of the cell, the disappearance of the Golgi material and the mitochondria. It terminates with the death of the secretory cell.

Intestinal epithelium.

1. The surface epithelium and the goblet cells are the basic type of cells.

2. The Golgi material in the epithelial cells lies above the nucleus. It shows marked changes both of a morphological and physico-chemical nature as soon as the cell comes into direct contact with food.

3. The mitochondria are in the form of filaments, rods and granules. A polar arrangement of the mito-chondria is a constant feature. When the cell is first stimulated by food, the mitochondria stain with great difficulty and then only faintly.

4. Secretory granules arise in close connection with the Golgi material and then move towards the glandular pole of the cell. Morphological changes of the Golgi material diminish and finally disappear towards the posterior part of the alimentary tract.

5. The Golgi material of the goblet cells lies above the nucleus. It enlarges greatly during secretory activity and decreases in mass during the final stage of secretion. /

of secretion.

6. The mitochondria are in the form of filaments, rods and granules. With the accumulation of secretory material the mitochondria collect on the border of the mucous mass and in the cytoplasm of the narrow part of the cell.

7. Secretory granules arise in close connection with the Golgi material. Secretory processes of these cells are autonomous and are not directly correlated with the digestion of food.

Salivary glands.

1. The Golgi material of the cells of the salivary glands hypertrophies greatly with the onset of secretion.

2. The mitochondria are in the form of rods and granules. Their polar arrangement marks the secretory phase. With the accumulation of secretion the mitochondria are pushed aside and are situated in the strips of cytoplasm between the mucous vesicles.

3. Secretory granules appear in close connection with the Golgi material. Feeding acts as a very weak stimulant causing evacuation of secretion. The evacuation of secretion is total and regeneration of the cell precedes the next secretory phase.

Pancreas.

1. The Golgi material of the cells of the pancreas lies near the acinar lumen. It enlarges during secretion /

secretion.

2. The mitochondria are thick filaments, rods and granules. Very few changes were observed in their morphology during secretion.

3. Secretory granules were observed in close proximity to the Golgi material. In the next phases they move towards the acinar lumen. Feeding acts as a stimulant causing evacuation of the secretory granules and the formation of new secretion. A complete evacuation of the secretory granules was not observed.

Liver.

1. The Golgi material lies at the glandular pole of the cell, close to the bile capillary. Its morphology changes considerably during the production of the new secretory granules.

2. The mitochondria are in the form of rods, filaments and granules. Granular forms predominate after 24 hours fasting, and rods and filaments are prevalent one hour after feeding. The polar arrangement of the mitochondria marks the secretory phase.

3. The secretory granules arise in connection with the Golgi material. Free secretory granules are not visible until six hours after feeding.

VII. CONCLUSIONS.

1. The Golgi material and the mitochondria were present in each group of cells investigated, and in every phase of activity.

2. In each type of cell examined the Golgi material underwent changes of form and disposition which were correlated with the phase of secretory activity.

3. The secretory granules were first visible in close connection with the Golgi material. It is concluded, therefore, that the Golgi material plays an important part in the formation of the secretory granules.

4. As only fixed material was used, it was not possible to determine if the mitochondria play a direct part in the formation of secretion.

VIII. REFERENCES TO LITERATURE.

1. Argeseanu, S. et May, R.M., 1938. "Études Différentielles sur la Cellule Embryonnaire et Adulte. I. Évolution des Constituants Cytoplasmique des Cellules de L'epithélium intestinal du Poulet (Gallus domesticus)", 'Arch. Anat. Micr.', Tom. 34, pp. 441- 448.

2. Argeseanu, S., 1934. "Les Constituants de la Cellule Intestinale des Ascaridies", 'C. R. Soc. Biol. Paris', Tom. 116, pp. 754- 756.

3. Arima, J., 1926. "Die Zytologische Untersuchungen der Bauchenweide bei den Embryonen. I. Mitteilung. Die Studien über das Pankreas", 'Folia Anat. Japon.' vol. 4, pp. 305- 332.

4. Arnold, G., 1912. "The Role of the Chondriosomes in the Cells of the Guinea Pig's Pancreas", 'Arch. Zellforsch.' Bd. 8, pp. 253- 271.

5. Baker, J.R., 1944. "Structure and Chemical Composition of the Golgi Elements", 'Quart. Journ. Micr. Sci.', vol. 85 (1) pp. 1- 71.

6. Beams, H.W., 1930. "Studies on the Vacuome and the Golgi Apparatus in the Acinar Cells of the Pancreas of the Rat", ' Anat. Rec.' vol. 45, pp. 137- 159.

7. Beams, H.W. and Goldsmith, J.B., 1930. "Golgi Bodies, Vacuome and Mitochondria in the Salivary Glands of the Chironomus Larva", 'Journ. Morph.', vol. 50, pp. 497- 511.

8. Beams, H.W. and King, R.L., 1932. "Notes on the Cytology of the Parietal Cells of the Stomach of the Rat", 'Anat. Rec.', vol. 53, pp. 31- 37.

9. Beams, H.W. and King, R.L., 1932. "The Architecture of the Parietal Cells of the Salivary Glands of the Grasshopper, With Special Reference to the Intracellular Canaliculi, Golgi Bodies, and Mitochondria", 'Journ. Morph.', vol. 53, pp. 223- 235.

10. Bowen, R.H., 1924. "On a Possible Relation Between /

Between the Golgi Apparatus and Secretory Products", 'Amer. Journ. Anat.', vol. 33, pp. 197- 215.

11. Bowen, R.H., 1926. "Studies on the Golgi Apparatus in Gland Cells", 'Quart. Journ. Micr. Sci.', vol. 70,
Part I. pp. 75- 112.
Part II. pp. 193- 216.
Part III.pp. 419- 550.

12. Bowen, R.H., 1929. "The Cytology og Glandular Secretion", 'Quart. Rev. Biol.', vol. 4,
pp. 300- 324.
pp. 484- 519.

13. Bufio, W., 1931. "L'Appareil de Golgi de la Cellule Exocrine du Pancréas dans Quelque Etats Physiologiques et Pathologiques", 'C. R. Soc. Biol. Paris', Tom. 106,
pp. 839- 841.

14. Calhoun, M.L., 1933. "The Microscopic Anatomy of the Digestive Tract of Gallus Domesticus", 'Iowa St. Coll. Journ. Sci.', vol. 7, (3) pp. 261- 382.

15. Clara, M., 1926. "Beiträge zur Kenntnis des Vogeldarmes", 'Zeitschf. Mikr. Anat. Forsch.
Bd. 6,
Teil II. pp. 1- 27.
Teil III. pp. 28- 54.
Teil IV. pp. 55- 75.
Teil V. pp. 256- 304.
Teil VI. pp. 305- 350.
Bd. 8,
Teil VII. pp. 22- 72.

16. Clark, J.A. and Hair, G.E., 1932. "Observations on the Mitochondria of the Hepatic Cell of the Frog in Normal and Hyperglycemic States", 'Zeitschrf. Zellforsch. Mikr. Anat.', Bd. 15, pp. 123- 130.

17. Claude, A., 1943. "The Constitution of Protoplasm", 'Science', vol. 97, pp. 451-456.

18. Corti, A., 1926. "Le Lacunome Révèle les Premières Modifications Structurales des Cellules Absorbantes de L'intestin au Cours de Leur Fonctionnement", 'Bull. Histol. Appl.' Tom. 3, pp.265- 272.

19. Covell, W.P., 1928. "A Microscopic Study of Pancreatic /

Pancreatic Secretion in the Living Animal", 'Anat. Rec.', vol. 40, pp. 213- 221.

20. Cowdry, E.V., 1924. "La Signification de L'Appareil Réticulaire Interne de Golgi en Physiologie Cellulaire", 'Bull. Histol. Appl.', Tom. 1, pp. 224- 240.

21. Cowdry, E.V., 1926. "Surface Film Theory of the Function of Mitochondria", 'Amer. Nat.', vol. 60, pp. 157- 165.

22. Cramer, W. and Ludford, R.J., 1925. "On Cellular Changes in Intestinal Fat Absorption", 'Journ. Physiol.', vol. 60, pp. 342- 346.

23. Cramer, W. and Ludford, R.J., 1926- 27. "On the Cellular Mechanism of Bile Secretion and Its Relation to the Golgi Apparatus of the Liver Cell", 'Journ. Physiol.', vol. 62, pp. 74- 86.

24. D'Agata, G., 1910. "Sur les Modifications de L'Appareil Reticulaire dans L'Epithelium de la Muqueude Gastrique", 'Arch. Ital. Biol.', Tom, 54, pp. 425- 428.

25. Dalton, A.J., 1933. "The Ontogenetic History of the Mitochondria and Golgi Network of the Hepatic Cell of the Chick", 'Anat. Rec.', vol. 58, pp. 321- 340.

26. Deineka, D., 1912. "Der Netzapparat von Golgi in Einigen Epithel- und Bindegewebszellen Während der Ruhe und Während der Teilung Derselben", 'Anat. Anzeiger', Bd. 41, pp. 289- 309.

27. Duthie, E.S., 1933. "Mucus Formation in Goblet Cells", 'Proc. Roy. Soc. B, vol. 113, pp. 459- 463.

28. Duthie, E.S., 1934. "Studies in the Secretion of the Pancreas and Salivary Glands", 'Proc. Roy. Soc., B, vol. 114, pp. 20- 47.

29. Eklöf, H., 1914. "Chondriosomenstudien an den Epithel- und Drüsenzellen des Magen-Darmkanals und den Ösophagus- Drüsenzellen bei Säugetieren", 'Anat. Hefte', Bd. 51, pp. 1- 227.

30. Florey, H., 1932. "Experimental Inflammation of the Colon, a) Relationship of Mucus Production /

Production in Goblet Cells to the Golgi Apparatus, b) Mitochondrial Changes," 'Brit. Journ. Exp. Path.', vol. 13, pp. 349- 359.

31. Gatenby, J.B., 1931. "The Prozymogen Granules (Vacuome) of R.R. Bensley in the Pseudotriton Pancreas, and the Modern Neutral-Red Cytology", 'Amer. Journ. Anat.', vol. 48, pp. 421- 472.

32. Gatenby, J.B., 1932. "Neutral Red Staining in Chironomus Salivary Gland Cells, and the So-called "Vacuome", 'Amer. Journ. Anat. vol. 51. pp. 253- 262.

33. Giroud, A., 1928. "Polarité Cellulaire at Appareil de Golgi", 'Bull. Histol. Appl.', Tom. 5, pp. 147- 152.

34. Gresson, R.A.R., 1934. "The Cytology of the Mid-gut and Hepatic Caeca of Periplaneta Orientalis", 'Quart. Journ. Micr. Sci.', vol. 77, pp. 317- 334.

35. Gresson, R.A.R., 1937. "A Study on Secretion and Cytoplasmic Inclusions in the Cells of the Salivary Glands of Tipula Paludosa", 'Zeitschrf. Zellforsch. Mikr. Anat.', Bd. 25, pp. 131- 142.

36. Guilliermond, A., 1941-42. "Données Actuelles sur la Signification Physiologique des Chondriosomes", 'Bull. Histol. Appl.' Tom. 18, pp. 93- 104.

37. Hall, E.M., and Mac Kay, E.M., 1933. "The Relation Between the Mitochondria and Glucose- Glycogen Equilibrium in the Liver", 'Amer. Journ. Path.' vol. 9, pp. 205- 220.

38. Hermanowa, S., 1932. "Appareil de Golgi et Chondriome dans les Cellules Épithéliales de L'Intestin Grêle chez Triton Cristatus Laur", 'C. R. Soc. Biol. Paris, vol. 110, pp. 801- 803.

39. Hibbard, H., 1942. "The Golgi Apparatus During Development in the Stomach of Gallus Domesticus", 'Journ. Morph.', vol. 70, pp. 121- 136.

40. Hirsch, G.C., 1931. "The Theory of Fields of Restitution /

Restitution, with Special Reference to the phenomena of Secretion", 'Biol. Rev.', vol. 6, pp. 88- 131.

41. Hirsch, G.C., 1932. "Die Lebendbeobachtungen der Restitution des Sekretes in Pankreas", 'Zeitschrf. Zellforsch. Mikr. Anat.', Bd. 15,
Teil I. pp. 36- 68.
Teil IV. pp. 290- 310.

42. Holmgren, E., 1902. "Einige Worte über das Trophospongium Verschiedener Zellarten", 'Anat. Anzeiger', Bd. 20, pp. 433- 440.

43. Honda, R., 1929. "The Golgi Apparatus in the Glandular Cells of the Submaxillary Gland of the Adult Albino Rat", 'Folia Anat. Japon.', vol. 7, pp. 203- 217.

44. Horning, E.S., 1929. "Studies on Mitochondria", 'Austral. Journ. Exp. Biol. Med. Sci.', vol. 6, pp. 11- 18.

45. Imai, Y., 1935. "On Golgi Apparatus of the Liver in Hepatic and Renal Disturbances", 'Jap. Journ. Gastroenterol.', vol. 7, pp. 202- 207.

46. Jacobs, W., 1929. "Untersuchungen über die Cytologie der Sekretbildung in der Mitteldarmdrüse von Astacus Leptodactylus", ' Zeitschrf. Zellforsch. Mikr. Anat.', Bd. 8, pp. 1- 62.

47. Kaywin, L., 1935. "A Cytological Study of the Digestive System of Anuran Larvae During Accelerated Metamorphosis", 'Anat. Rec.', vol. 64, pp. 413- 437.

48. Kirkman, H. and Severinghaus, A.E., 1938. "A Review on the Golgi Apparatus", 'Anat. Rec.', vol. 70,
Part I. pp. 413- 433.
Part II. pp. 557- 575.
vol. 71,
Part III. pp. 79- 103.

49. Kopsch, F., 1926. "Das Binnengerüst in den Zellen Einiger Organe des Menschen", 'Zeitschrf. Mikr. Anat. Forsch.', Bd. 5, pp. 221- 284.

50. Lim, R.K.S. and Ma, W.C., 1927. "Mitochondrial Changes /

Changes in the Cells of the Gastric Glands in Relation to Activity", ' Quart. Journ. Exp. Physiol.', vol. 16, pp. 87- 110.

51. Ling, M.S., Liu, A.C. And Lim, R.K.S., 1928. "The Lipoid Metabolism of the Stomach and Its Relation to the Mitochondria-Golgi Complex", 'Chin. Journ. Physiol.', vol. 3, pp. 305- 328.

52. Liu, A.C., 1930. "The Mitochondria-Golgi Complex of the Columnar Epithelium of the Small Intestine During Absorption", 'Chin. Journ. Physiol.', vol. 4, pp. 359- 364.

53. Ludford, R.J., 1928. "The Vital Staining of Normal and Malignant Cells", 'Proc. Roy. Soc.' B, vol. 103, Part I. pp. 288- 301. vol. 107, Part III. pp. 101- 114.

54. Ma, W.C., 1924. "The Changes in the Pancreatic Cell of the Guinea Pig During Inanition and Refeeding", 'Anat. Rec.', vol. 27, pp. 47- 60.

55. Ma, W.C., 1928. "Relation of the Mitochondria-Golgi Complex to Secretion", 'Chin. Journ. Physiol.', vol. 2, pp. 247- 254.

56. Ma, W.C., Lim, R.K.S. and Liu, A.C., 1927. "Changes in the Golgi Apparatus of the Gastric Gland Cells in Relation to Activity", 'Chin. Journ. Physiol.', vol. 1, pp. 305- 330.

57. Martin, J.F., Croizat, P. et Guichard, A., 1930. "Le Chondriome Hépatique dans L'amaigressement par Jeûne Prolongé", 'Bull. Histol. Appl.', Tom. 7, pp. 111- 117.

58. Meerssemann, F., 1939. "Le Chondriome de la Cellule Hépatique chez le Cobaye Normal", 'Bull. Histol. Appl.', Tom. 16, pp. 215- 228.

59. Miller, S.P., 1922. "Effect of Various Types of Inanition Upon the Mitochondria in the Gastro- Intestinal Epithelium and in the Pancreas of the Albino Rat", 'Anat. Rec.', Vol. 23, pp. 205- 210.

60. Mislawski, N., 1912. "Uber das Chondriom der Pankreaszellen", 'Arch. Mikr. Anat.', Bd. 81, pp. 394- 429.

61. Moley, H.G. and Smith, J.H., 1930. "Mitochondria in the Submaxillary Gland of the Rabbit, with Particular Reference to Nerve Stimulation", 'Anat. Rec.', vol. 45, pp. 337- 344.

62. Morelle, J., 1924. "La Substance de Golgi dans les Cellules Pancreatiques des Vertébrés", 'C. R. Soc. Biol. Paris', Tom. 91, pp. 1173- 1175.

63. Muggia, G. e Masuell, L., 1932. "La Citologia Della Epatica in Rapporto Alla Qualita Dell' Alimento Introdotto", 'Zeitschrf. Zellforshc. Mikr. Anat.', Bd. 16, pp. 659- 673.

64. Mc Cudry, M.B.D., 1939. "Mitochondria in Liver Cells of Fed and Starved Salamanders", 'Journ. Morph.', vol. 64, pp. 9- 30.

65. Mc Cudry, M.B.D., 1940. "The Effect of Growth and Nutrition on Mitochondria in Liver Cells of Fundulus Heteroclitus", 'Biol. Bull.', vol. 79, pp. 252- 254.

66. Mc A. Kater, J., 1931. "Variations in the Mitochondria of the Hepatic Cell in Relation to Alterations of the Glycogen-Glucose Equilibrium", 'Anat. Rec.', vol. 49, pp. 277- 287.

67. Mc A. Kater, J., 1933. "Comparative and Experimental Studies on the Cytology of the Liver", 'Zeitschrf. Zellforsch. Mikr. Anat.', Bd. 17, pp. 217- 246.

68. Mc A. Kater, J.,1937. "The Mitochondria of the Hepatic Cell During Cholagogenic Stimulation", 'Zeitschrf. Zellforsch. Mikr. Anat.', Bd. 25, pp. 127- 130.

69. Nassonov, D., 1923-24. "Das Golgische Binnennetz und Seine Beziehungen zu der Sekretion", ' Arch. Mikr. Anat.', Bd. 97, pp. 136- 186. Bd. 100, pp. 433- 472.

70. Noël, R. et Pallot, G., 1933. "Recherches sur le Chondriome de la Cellule Hépatique des/

des Mammiféres", 'Bull. Histol. Appl.', Tom. 10, Part I. pp. 169- 186. Tom. 11, Part II. pp. 75- 96. Part III. pp. 115- 120.

71. Okanishi, J., 1933. "Studies on the Mitochondria and Metachondria of the Gastric Gland Cells", 'Jap. Journ. Exp. Med.', vol. 11, Part I. pp. 407- 419. Part II. pp. 439- 446.

72. Parat, M.M. et Painlevé, J., 1925. "Rôle du Vacuome (Appareil de Golgi) et du Chondriome dans la Formation des Grains de Sécrétion", 'C. R. Soc. Biol. Paris', Tom. 92, pp. 65- 67.

73. Pfuhl, W. und Dienstbach, O., 1938. "Die Speicherung und Verarbeitung von Kolloiden Farbstoffen, Pigmenten und Lipoiden in der Golgi- Substantz der Leberzellen", 'Zeitschrf. Anat. Entwicklungsgesch.', Bd. 108, pp. 260- 282.

74. Pollister, A.W., 1932. "The Cytology of the Liver of Amphiuma", 'Amer. Journ. Anat.', vol. 50, pp. 179- 192.

75. Pollister, A.W., 1938-40. "The Structure of the Golgi Apparatus in the Tissues of Amphibia", 'Quart. Journ. Micr. Sci.', vol. 81, pp. 235- 271.

76. Pollister, A.W., 1941. "Mitochondrial Orientation and Molecular Pattern", 'Physiol. Zoöl.', vol. 14, pp. 268- 279.

77. Ries, E., 1935. "Zur Histologie des Mäusepankreas nach Lebendbeobachtung, Vitalfärbung und Stufenuntersuchung", 'Zeitschrf. Zellforch. Mikr. Anat.', Bd. 22, pp. 523- 585.

78. Saguchi, S., 1919-20. "Studies on the Glandular Cells of the Frog's Pancreas", 'Amer. Journ. Anat.', vol. 26, pp. 347- 421.

79. Saito, R., 1933. "Study on Mitochondria and Metachondria of Intestinal Epithelial Cells", 'Jap. Journ. Exp. Med.', vol. 11, pp. 397- 405.

80. Sawada, Y., 1935. "Studies on the Mitochondria, Metachondria /

Metachondria on Golgi's Apparatus and on the Silvergranules of Lieberkühns Gland Cells of the Intestine of the White Rat", 'Jap. Journ. Exp. Med.', vol. 13, pp. 441- 455.

81. Steffens, H.W., 1941. "An Experimental Study of the Mitochondria in the Hepatic Cells of the White Rat", 'Anat. Rec.', vol. 81, pp. 243- 260.

82. Subramaniam, M.K., 1938. "Studies on the Structure of the Golgi Apparatus", 'Proc. Indian Acad. Sci.' B., vol. 7, Part II. pp. 80- 103. Part III. pp. 125- 131.

83. Tschassovnikow, N., 1927. "Uber den Gang des Sekretions Prozesses in den Zellen des Magendeckepithels bei Einigen Amphibien und Säugern", 'Zeitschrf. Zellforsch. Mikr. Anat.', Bd. 5, pp. 680- 704.

84. Suzuki, J., 1935. "Studies on Mitochondria and Metachondria of the Gland Cell of the Pancreas", 'Jap. Journ. Exp. Med.', vol. 13, pp. 285- 305.

85. Weatherford, H.L., 1932. "The Golgi Apparatus and Vital Staining of the Amphibian and Reptilian Liver", 'Zeitschrf. Zellforsch. Mikr. Anat.', Bd. 15, pp. 343- 372.

86. Weiner, P., 1928. "Über Fettablagerung und Fettresorption im Darm", 'Zeitschrf. Zellforsch. Mikr. Anat. Forsch.', Bd. 13, pp. 197- 268.

87. Williams, M.A., 1943. "Mitochondria in the Intestinal Epithelial Cells of Starved and Fed Salamanders", 'Anat. Rec.', vol. 85, pp. 195- 205.

88. Woodruff, B.H., 1933-34. "Studies of the Epithelium Lining the Caeca and Mid-gut in the Grasshopper," 'Journ. Morph.', vol. 55, pp. 53- 74
